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W. B. BRIERLEY

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D. WARD CUTLER

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STUDIES ON POTATO VIRUS DISEASES

IV. FURTHER EXPERIMENTS WITH POTATO MOSAIC

BY KENNETH M. SMITH, D.Sc.

(*Potato Virus Research Station, School of Agriculture, Cambridge.*)

(With Plates I–V.)

CONTENTS.

1. INTRODUCTION.

THE fact that some at least of the potato virus diseases are spread from plant to plant through the agency of insects is no longer open to doubt, but information as to which of the many insect species attacking the potato are capable of disseminating the virus is scanty. Some evidence has already been presented by the writer (8) that the most efficient carrier of mosaic disease in Great Britain is the aphis *Myzus persicae* Sulz. and possibly also another species of aphis, *Macrosiphum gei* Koch. Further evidence of successful transmission by the former aphis is offered in the ensuing communication, together with an account of some negative experiments with other potato insects.

The main purport of this paper, however, is to describe in detail experiments with the virus of potato mosaic upon another Solanaceous

host, *i.e.* the tobacco plant, illustrating the disease it produces in that host, and the changes that have occurred in the response of the potato plant to the mosaic virus after its passage through the tobacco plant.

Grateful acknowledgment is due to Miss F. E. Hawkes, who by her entirely voluntary assistance in the mornings during the summer of 1928 relieved the writer of much necessary routine work.

2. MATERIAL AND METHODS.

Technique of insect infection of potato and tobacco plants.

(*a*) *Potato.* With the exception of the insect infections of the potato haulm performed in 1926 in the open under insect-proof cages, all the work detailed in this communication was carried out in the insect-proof glass-houses at the Potato Virus Research Station, School of Agriculture, Cambridge.

To infect the insects used with the mosaic virus they were colonised upon mosaic potato plants growing in pots, either under large glass cylinders with canvas tops or in rectangular wooden cages also with canvas tops and with glass in three out of the four sides. For aphis infection of the haulms of healthy potatoes the same type of cage was used, the bottoms of the cages being covered with sand as an additional safeguard against insect escape. For insect infection of the sprouted half tuber, two methods of procedure were tried. The first method consisted in placing the sprouted half tuber in an ordinary two-pound glass jam jar, fitted with a muslin top secured with a rubber band, and introducing the insects on to the sprouts. This was found unsatisfactory and soon abandoned, as the half tuber tended either to rot or dry up and the sprouts thus to become unsuitable for the insects to feed upon them. The second method of sprout infection was definitely satisfactory, and consisted in planting the half tuber in a pot with the sprouts uncovered by soil, a glass lamp chimney of the "hurricane" or stable lamp type was then placed over the sprouts and the insects introduced.

The rim of the lamp glass allowed of a canvas top, held in place by a rubber band, being fixed, while the lower rim of the glass was pressed into the soil, thus providing an insect-proof cage. By this method ideal conditions for plant infection were obtained, *i.e.* a young and rapidly growing plant, together with the necessary humidity for the insect, an important point in the case of the aphis. After the requisite time for infection of the plant had elapsed, the lamp chimney was removed, the young shoot sprayed with nicotine and soap and allowed to grow to maturity.

(*b*) *Tobacco.* The tobacco plants, used either as the source of infection or as healthy plants to be infected, were grown in pots under the glass cylinders and glass-sided cages already mentioned. It was necessary to use small seedlings, both for ease of infection and because the later development of sticky hairs prevented the colonisation of the aphides (the only insects so far used in tobacco infection). After the necessary time had elapsed the plants were sprayed with nicotine and soap.

Origin of plants used.

The healthy potato plants were mostly of two varieties, Arran Victory and President. The former were part of the writer's healthy stock, grown for 2 to 3 years under insect-proof conditions, and the latter were tested tubers kindly given by Dr R. N. Salaman. The mosaic potato plants were of the same two varieties, and had also been grown for 2 years under the same conditions.

The tobacco plants were of two varieties, White Burley and Virginia, grown from seed supplied by Messrs Sutton of Reading.

Origin of insects used.

The aphis, *Myzus persicae*, so largely used in these experiments, was from a stock which had been breeding continuously for three years upon non-Solanaceous plant hosts such as spinach, radish, cabbage and chrysanthemum, etc. The other insects used were collected mostly from nettle which is an alternate host for the majority of potato-inhabiting insects.

3. EXPERIMENTAL TRANSMISSION OF POTATO MOSAIC BY INSECTS.

(*a*) *Haulm infections*, 1926.

Two plots of land near Manchester were used for these experiments, one in the experimental grounds of the University, and the other in the grounds of the Shirley Institute at Didsbury, by kind permission of the director. The plants used in one plot were part of the writer's stock of healthy Arran Victory, and those used in the other were Tinwald Perfection, Scotch "seed." Each healthy tuber used in these experiments was divided into two halves, which were planted under separate and similar insect-proof cages which have been described elsewhere(8). One half formed the plant to be infected, and the other half acted as a control, two plants per insect species were used in each plot. When the experimental plants were about 10 in. high they were colonised with the various insects under test, which had previously fed for a minimum

period of a week upon a mosaic potato plant, variety President. A separate mosaic plant was used to infect each species of insect. The following insects were used:

HEMIPTERA

Capsidae

Lygus pabulinus Linn.
Calocoris bipunctatus Fab. (*norvegicus*)

Leaf-hoppers

Zygina pallidifrons Edw.
Eupteryx auratus Liv.

Aphididae

Myzus persicae Sulz.
Macrosiphum gei Koch.
Myzus circumflexus Buckt.

Aleurodidae

Asterochiton vaporariorum Westw.

COLEOPTERA

Psylliodes affinis.

All these insects were successfully colonised, and the plants grew normally with the exception of the two Arran Victory potatoes colonised with the aphis *Macrosiphum gei*, which died down prematurely.

Results of 1926 *haulm infections.* All the plants in Exp. 1 (Arran Victory) with their controls remained healthy during 1926. Exp. 2 (Tinwald Perfection) had to be discarded owing to the development of mosaic in the control plants. The tubers resulting from the Arran Victory experiment were carefully harvested from both experimental and control plants and kept in glass jars with close-fitting canvas tops until the spring of 1927, when they were brought to Cambridge and planted in the insect-proof glass-house of the Potato Virus Research Station. The progeny of both experimental and control plants remained healthy throughout 1927. The tubers resulting from these plants were harvested and planted under the same conditions as in 1927. This time mosaic of a well-marked type developed in all the plants originating from one of the potatoes colonised with *Myzus persicae* in 1926. The controls to this experiment and all the other insect experiments and controls remained healthy. Although some tubers were formed, the experiment with the aphis *M. gei* cannot be considered owing to the premature failure of the haulms. It would appear from the results of this experiment that it is possible for the mosaic virus to lie latent, at any rate in the variety Arran Victory. There seems no explanation other than that the aphis *M. persicae* had infected the plant in 1926 and the virus had remained dormant till 1928, as there had been no chance of any extraneous infection between that time and the development of mosaic symptoms.

That the aphis, *M. persicae*, does actually pick up the virus of potato mosaic with regularity has been proved by the writer, and will be dealt with later in this paper. Although it must be regarded as a possibility, the phenomenon of temperature masking hardly seems adequate as an explanation of this belated development of mosaic, as other plants under exactly similar conditions showed the mosaic symptoms quite normally. As the progeny of the plants, colonised in 1926 with the other species of insects, remained healthy throughout 1928, it would appear that these insects had failed to transmit the virus. After several years' experience with potatoes under insect-proof cages the writer has formed the opinion that absolutely critical experimental work with the insect transmission of potato virus diseases cannot be performed under cages out of doors, and this type of experiment has therefore been abandoned.

(*b*) *Sprout infections*, 1927.

The experiments outlined in the preceding paragraphs were repeated in 1927 using the same insects, all the work being carried out in the insect-proof greenhouse. In this case, however, sprouted half tubers were used, and the insects, after feeding upon a mosaic Arran Victory plant, were colonised on the sprouts instead of on the growing plant. It was hoped thereby that the symptoms of mosaic disease, if transmitted, would show in the current season. The sprouted half tubers, after infection with the insects, together with the controls, were grown to maturity in the glass-house. All remained healthy with the exception of a certain percentage, the controls of which also developed mosaic; these were immediately discarded.

Owing to a shortage of healthy tubers the writer was compelled to perform some of the experiments with "seed" tubers bought from Scotland, but from this and other experiences it is not considered possible to perform experiments with potato mosaic except with tubers, the history of which is accurately known.

The progeny of the other experimental plants were grown in 1928, again with negative results, except in the case of *M. persicae*, where three plants out of twelve showed mosaic symptoms.

In this series of sprout infections many factors were varied, such as the times on source of infection and healthy sprout, temperature, and age of insect used, such as adult and larval forms.

4. ATTEMPTED TRANSMISSION OF POTATO MOSAIC BY MEANS OF INOCULATION OF HEALTHY POTATOES WITH THE TISSUES OF PRESUMABLY INFECTIVE INSECTS.

Exp. 1. Attempted infection of healthy potato plants by means of the body juices of mosaic-carrying aphides.

About 8 c.c. in bulk of the aphis, *M. persicae*, which had lived only upon a mosaic potato plant (Arran Victory) were triturated *en masse* in a mortar, under sterile conditions, with 20 c.c. of sterile water. The resulting fluid was filtered through a piece of fine muslin to remove the insect fragments, and some of the clear fluid thus obtained was apportioned into six small sterile glass tubes. Shoots were cut from six known healthy potato plants, Arran Victory and President, and placed each in a tube containing the aphis extract for 48 hours. At the end of that time each shoot had absorbed practically all the fluid in its tube. The shoots were then grafted back each on to its own plant. Union was made in every case and the shoots grew normally, no symptoms of mosaic developing upon either shoot or plant. A number of other healthy shoots were allowed to absorb the remaining aphis extract and were then plunged in pots where they put out roots and made plants. These remained healthy. The progeny of all the plants used in the above experiment were grown the following year and all produced healthy plants.

Exp. 2. Insertion into healthy potato sprouts of the salivary glands of two species of Capsid bugs (*Lygus pabulinus* and *Calocoris bipunctatus*) which had been bred upon mosaic potato plants.

These experiments were carried out during the two years 1926–1927. In the first year the salivary glands of twenty-five specimens of each of the two species of Capsid bugs were extracted and inserted with a needle into the sprouts of twelve healthy half tubers, each with a half tuber control. The half tubers were then potted up and grown under insect-proof conditions. This experiment was entirely negative, both inoculated and control half tubers producing healthy plants. It is noteworthy that the salivary glands of these two bugs produced necrotic lesions in the sprouts at the point of insertion.

In 1927 the procedure was altered slightly. The salivary glands of the two species of bugs which had fed, as before, on mosaic potato plants were extracted and triturated in a mortar under sterile conditions with 5 c.c. of sterile water. Shoots from known healthy Arran Victory and President plants were cut off and allowed to suck up this extract as in Exp. 1. They were then grafted back on to their respective plants, where they grew normally. No sign of mosaic appeared either in the plant itself

or on the grafted shoot, nor did the resulting tubers produce diseased plants when grown the following year.

From these two experiments, and others not described, it would appear impossible to infect potato plants with mosaic disease by means of inoculation with the body juices or salivary glands of insects which have been bred upon mosaic potato plants.

5. EXPERIMENTAL TRANSMISSION OF THE VIRUS OF POTATO MOSAIC BETWEEN POTATO AND TOBACCO PLANTS.

After so much negative work with the attempted transmission of potato mosaic by means of insects, it occurred to the writer that if some other Solanaceous plant could be found which might more easily respond to the mosaic virus some further information might be obtained. If such a plant were infected it would at least act as an indicator in the sense that it would show whether or not the aphis was actually picking up the virus of potato mosaic. The plant selected for this work was the tobacco, two varieties being used, White Burley and Virginia. The following preliminary experiment which consisted of three parts was performed. Firstly, twelve half tubers of healthy Arran Victory, which were potted up and showing sprouts about 2 in. above the soil, were colonised with the aphis *M. persicae* which had been bred upon a mosaic Arran Victory plant. This aphis was chosen, judging from the writer's experience, as being the most likely insect to give positive results. Secondly, aphides from the same mosaic Arran Victory plant were colonised on six young tobacco plants, three White Burley, three Virginia. The third part of the experiment consisted of inoculations by means of a sterile needle, with juice from the same mosaic potato, on to six tobacco plants, three of each variety. The results of the experiment were as follows: the twelve Arran Victory potato plants showed no symptoms and were presumably still uninfected, the three White Burley tobacco plants colonised with the aphides developed a very marked spotting combined with mottling, the three Virginia plants colonised with the aphis developed no symptoms, while five out of the six tobacco plants inoculated by means of the needle developed some rather remarkable symptoms. Two out of the three Virginias showed a number of well-marked concentric rings, each with a central spot (Plate I, fig. 1), the walls of the rings being sharply cut, necrotic, and whitish in colour. The manifestations of this "ringspot" disease consisted either of two concentric rings, two pairs of concentric rings (Plate I, fig. 1), with a central spot, or sometimes single rings only without the spot. The three White Burley tobaccos showed a disease similar in general appearance,

but differing in the fact that instead of sharply cut concentric rings, the disease showed itself in the form of large numbers of round necrotic spots, some of which approached a ring-like form but seldom showing the very clear-cut rings appearing in Virginia.

This experiment was repeated with the tobaccos only, leaving out the aphis infection of the healthy Arran Victory plants, and using both aphides and inoculum from the original mosaic Arran Victory. The results of this second experiment were the same as those in the first.

From these two preliminary experiments a number of lines of enquiry were developed, the results of which, together with details of the experiments performed, are presented in the remainder of this paper.

To avoid the confusion likely to arise in describing numbers of cross-inoculation studies, the work has been grouped into the following sections, each series of experiments being dealt with separately.

Section 1. Inoculation of healthy tobacco plants of two varieties with the virus of potato mosaic.

(*a*) By needle.

(*b*) By aphis.

Section 2. Inoculation of healthy potato plants with the virus of various forms of tobacco ringspot.

(*a*) By needle.

(*b*) By aphis.

Section 3. Are the varying symptoms produced in tobacco by needle inoculation with potato mosaic manifestations of the same disease?

Section 4. Comparison of the symptoms produced in tobacco by aphis and needle inoculation respectively from the same mosaic potato.

Section 5. Transmission of tobacco ringspot to healthy tobacco.

(*a*) By needle.

(*b*) By aphis.

Section 6. Transmission of potato "ringspot" or intensified mosaic to healthy potato plants.

(*a*) By needle.

(*b*) By grafting.

(*c*) By aphis.

Section 7. What is the effect on the virus of tobacco ringspot of progressive inoculations through successive generations of tobacco plants?

Section 8. What is the effect on the virus of potato "ringspot" or intensified mosaic of progressive inoculations through successive generations of potato plants?

Section 9. Inoculation of healthy tobacco plants with the virus of potato "ringspot" or intensified mosaic.

(*a*) By needle.

(*b*) By aphis.

Section 10. Inoculation of healthy tobacco plants with the juice of known healthy Arran Victory potatoes.

Section 11. Effect of temperature on the symptoms of ringspot.

(*a*) In potato.

(*b*) In tobacco.

Filtration of the virus.

Section 12. Inoculation of tobacco plants with virus combinations of which potato mosaic is a constituent part.

Section 13. Needle inoculation of plants other than tobacco or potato with the virus of tobacco ringspot.

(*a*) Solanaceae.

(*b*) Other plants.

SECTION 1.

Inoculation of healthy tobacco plants of two varieties with the virus of potato mosaic:

(*a*) *By needle.*

The mosaic virus was obtained from infected potatoes mostly of the variety Arran Victory, the history of which was known. These potatoes had been grown for two years under insect-proof conditions, and so far as could be ascertained were affected only with mosaic, the symptoms being the usual mottling associated with that disease. Leaves from such potatoes were ground up in a mortar under sterile conditions without the addition of water or with just sufficient to form a thick fluid of the consistency to allow of easy handling with a needle. This fluid was then scratched into the lamina and petiole of the tobacco leaf, while an incision was also usually made in the stem and some mosaic tissue inserted. It is important to use very small seedlings as it was found much more difficult to infect larger plants or those which were in any degree pot-bound.

Development of ringspot in the inoculated tobacco plants.

(*a*) *Virginia.* The development of symptoms of ringspot in both Virginia and White Burley is not always constant, even differing in a number of plants treated under identical conditions with the same inoculum. As a general rule, however, a mottling develops as the young leaves grow; this may disappear later, its place being taken by the curious

rings which give the disease its name, or it may persist in a characteristic spot-necrosis form. These rings in this particular variety may be of two kinds, either of a nebulous type somewhat suggestive of the watermark in a sheet of paper (Plate I, fig. 4) or of the concentric double ring with or without a central spot, and with clearly cut whitish necrotic walls (Plate I, fig. 1). The nebulous type of ring often appears first, later its place may be taken by the concentric ring, which is especially characteristic of Virginia tobacco, or the two may exist together. In one case the same inoculum produced the necrotic ring in one plant and the nebulous ring in another. As a rule, however, the necrotic concentric ring is the chief and may be the only symptom in this variety. Very often an infected Virginia plant presents a normal appearance except that in the centre of one leaf may appear a single concentric ring.

As the infected Virginia plant grows, new rings form on the young leaves; the old rings tend to lose their clear-cut character and to merge into necrotic spots, or they may persist for a time in the form of rings with reddish necrotic walls. When the plant is full grown and after the elapse of a month or two from the date of inoculation, the rings have mostly disappeared and the leaves present either a uniform mottled appearance, or else a curious pattern of wavy lines of darker green which follow the veins of the leaf (Plate III, fig. 2). That the virus is still active in the mature plant has been proved by the inoculation of the juice into fresh seedlings. As compared with White Burley tobacco, the Virginia variety exhibits a very strong resistance to ringspot. In all the writer's inoculation experiments, both by needle and aphis, but particularly the latter, this varietal resistance is borne out.

In addition the disease is definitely less harmful to Virginia than to White Burley. As already stated a "ringspot" Virginia plant may be normal in appearance except for isolated double rings scattered sparingly over the leaf surface. Nevertheless, this resistance is only comparative, and ringspot is easily spread from plant to plant by needle inoculation.

(*b*) *White Burley.* The first developments of ringspot in White Burley are somewhat similar to those in the foregoing variety; the rings, however, very rapidly lose their individuality and merge into necrotic spots and blotches (Plate II, fig. 1). Occasionally the symptoms may appear as patches, consisting of half double ring and half necrotic spot. The "spot necrosis" type of disease is decidedly characteristic of White Burley. In this variety the disease has a decidedly deleterious effect upon the health of the plant, as a rule the White Burley infected with ringspot is stunted and poorly developed. It often shows a curious one-sided

growth, and may die without reaching maturity. The different manifestations of ringspot in tobacco are shown in Plates I and II. In addition to the two types of rings, there is the spot necrosis, the lines of darker green following the veins, the so-called "clearing of the veins" often associated with the early development of the disease, and the aphis-induced mottling described in the next paragraph. In all, six series of experiments were successful in inducing ringspot in tobacco by means of needle inoculation with potato mosaic, and one unsuccessful. Six different mosaic Arran Victory plants were used as the sources of inoculum in the series during the years 1927–1928. Inoculations with juice from one potato of another variety exhibiting mosaic also produced typical ringspot.

The time elapsing before the appearance of symptoms in the inoculated tobacco plants appears to be governed largely by the temperature, and incubation periods ranged from 34 days during the winter to 12 days or less during July and August.

(*b*) *By aphis.*

The series of mosaic Arran Victory potato plants used in the needle inoculations were also used for the aphis transmissions, and the species employed was *Myzus persicae* Sulz. After feeding upon the mosaic foliage for a minimum period of one week the insects were colonised upon young tobacco seedlings. It is only possible to feed aphides upon these two varieties of tobacco while the plants are in the seedling stage, after this the development of sticky hairs renders aphis colonisation impossible. In all, eight series of experiments with aphis transmission of potato mosaic to White Burley and Virginia tobacco were carried out. Of these, six were successful in White Burley and two in Virginia, added proof of the greater resistance in the latter. The disease produced in the tobacco by the aphis was distinctly characteristic. It resembled in some ways the spot necrosis type produced in White Burley by needle inoculation, but yet had a distinctive character. The first manifestation consists of a "clearing of the veins." In this the veins stand out more than is normal from the rest of the leaf; this later develops into a well-marked mottle accompanied by numbers of lighter-coloured spots. So far the writer has not succeeded in producing definite concentric rings in tobacco by means of the aphis, but in one series the aphis produced the nebulous watermark type of ring. In old plants this "spot mottle" has sometimes merged into the dark green lines following the leaf veins, which also may be found in mature plants with needle-induced ringspot (Plate III, fig. 1).

The times elapsing between the date of first infection of the tobacco plant with aphis and the appearance of the symptoms varied between 44 days in January and 15 days in June. By the regular production of a disease in tobacco by means of *Myzus persicae* from mosaic potato, evidence is presented that the aphis does actually pick up the mosaic virus from the infected potato plant. The reasons for the frequent failure of the aphis to transmit the virus, once picked up, to healthy potatoes under the writer's conditions are yet to seek. It is of interest to find from the foregoing experiments that the virus of potato mosaic has apparently undergone some slight change in the body of the insect. At any rate the symptoms of the disease produced in tobacco by aphis from mosaic potato are quite distinct from the symptoms produced by needle inoculation from the same source. That this change is only slight is proved by the experiments detailed in Section 4, which show that the aphis mottle of tobacco and the true ringspot both produce identical symptoms upon healthy potato.

Section 2.

Inoculation of healthy potato plants with the virus of various forms of tobacco ringspot:

(*a*) *By needle.*

A large number of inoculations into healthy potato plants (varieties Arran Victory and President) were made with tobacco ringspot in all its manifestations. The needle inoculations were carried out in the same manner as those into tobacco, and scratches were made into the potato leaf-blade and petiole, and some tissue was also introduced into a stem incision. Altogether fifteen series of experiments, using about sixty plants, were made. Symptoms commenced to develop in the inoculated potato plant in periods ranging from 7 to 21 days at temperatures below 80° F. Not one of the sixty plants inoculated failed to develop disease. The disease thus produced may be compared to the original potato mosaic before its passage through the tobacco, but differing in the intensification of the mosaic mottling and above all in its enormously increased infective power. The development of this intensified or "ringspot" form of mosaic is more constant in potato than in tobacco. (For want of a better term, this intensified mosaic in the potato will be referred to as "ringspot" mosaic for convenience and not because ringspots are formed upon the potato leaf.) The first indication is the formation of well-marked spots of light green or yellow upon the youngest leaves. These spots increase in size and number, spreading over almost the entire plant with the excep-

tion of the older leaves, presenting a most brilliant mosaic mottling. The younger leaves also sometimes lose their regular outline and become crinkled and distorted. At the same time as the mosaic mottle is developing on the younger leaves, large numbers of fine necrotic spots, blackish red in colour, appear on the older leaves (Plate V, fig. 1), accompanied by a few larger lesions of the same character. These necrotic spots may also occur on the young leaves, but are more usually confined to the lower leaves. After about a week or 10 days the small necrotic spots disappear, and no new lesions are developed. The characteristic mottling, however, persists for the remainder of the life of the plant. In some cases this disease appears to have a temporarily arresting effect upon the development of the plant. Instances were observed where the potato plant remained stationary for several weeks; during that period the mosaic mottling became fixed and necrotic, the mottled areas remaining as white dead patches. After a period of 4 or 5 weeks the plant would start into active growth again, but fresh mottling of a very marked character would develop on each new leaf as formed, showing that the virus was still vigorous within the plant. In some potato plants which have been infected for 5 or 6 weeks and which have grown normally, the writer has noticed a tendency of the "ringspot" mosaic to revert, so far as the mottling goes, to the appearance of the original mosaic before its passage through the tobacco, quite apart from any suppression of symptoms by temperature. Whether the virus is actually reverting to it original condition or whether, as seems possible, the symptoms flag owing to the approaching maturity of the plant is not known at present. The writer, however, has often noticed in ordinary potato mosaic the disappearance of the symptoms with continued growth of the plant. The disease produced in the healthy potato by inoculation with tobacco ringspot appeared the same whatever the manifestation of the tobacco disease at the time of inoculation, *i.e.* necrotic rings, nebulous rings, mottle, etc. Two experiments were also performed of inoculating healthy potato plants with the virus of tobacco ringspot which showed some increase in virulence after progressive inoculation through succeeding White Burley tobacco plants. The disease developed in the potato in both experiments in 12 days. In five out of six plants the symptoms were those characteristic of the intensified form of mosaic, but in the sixth (President) the lesions were of an unusually severe type.

(*b*) *By aphis.*

Although the virus of the ringspot or intensified mosaic in potato is so infectious that a needle scratch rarely fails to pass over the disease to healthy plants, it has not adapted itself proportionately to transmission by the aphis. Out of seven series of transmission experiments with the aphis *M. persicae*, involving a total of fifty plants, only two plants (Plate IV, fig. 2), with a possible third, became infected with the disease in periods of 14 days and 24 days from the date of first infection with the aphis.

Section 3.

Are the varying symptoms produced in tobacco by needle inoculation with potato mosaic manifestations of the same disease?

In order to determine whether this difference in symptoms was due merely to a difference in varietal response to the virus, or whether there existed one or more diseases, the following cross-inoculations were made. Healthy Virginia plants were inoculated with the juice from White Burley tobacco plants showing the characteristic spot necrosis. In 15 days the Virginia plants showed the typical necrotic double ring. Healthy White Burley plants were inoculated with juice from Virginia showing necrotic rings. In 16 days the White Burley plants developed a typical spot necrosis. These cross-inoculations offer fair evidence that the varying symptoms are varietal presentations of the same disease. With regard to the other manifestations of ringspot in both tobaccos, such as clearing of the veins, the different mottlings, etc., it has often been found that one type will grade into another, and this together with the evidence presented in Section 2, where both forms are shown to produce identical symptoms in healthy potato plants, seems sufficient justification for supposing that there is only one disease under consideration, more especially when it is realised that all have a common source in mosaic potato. Diagnosis of virus disease from the symptoms, however, is notoriously unreliable, and the matter could probably only be settled by a study of the physical properties of the virus.

Section 4.

Comparison of the symptoms produced in tobacco by aphis and needle inoculation respectively from the same mosaic potato.

Five series of needle inoculations into healthy potato plants with this aphis-induced mottle were carried out. In all, seventeen plants of Arran Victory and President were inoculated; in each case the potato plants

developed an intensified form of mosaic in no way differing, so far as the symptomatology of the disease can indicate, from the disease produced by needle inoculation into healthy potatoes of the needle-induced tobacco ringspot. Added to this is the fact that, although the aphis-induced disease in tobacco has not yet shown in the concentric ringspot form, the ringspot form and the aphis-mottle have been found to merge into similar symptoms in mature plants. For these reasons, and bearing in mind that the same mosaic potato plant was the source of infection, the writer considers that the aphis-mottle and the various forms of ringspot in tobacco are all manifestations of the same virus disease.

SECTION 5.

Transmission of tobacco ringspot to healthy tobacco:

(*a*) *By needle.*

Ringspot of tobacco is very easily transmissible to healthy tobacco plants by means of needle inoculation, more easily in the case of White Burley than in Virginia. Symptoms in inoculated plants develop in 7 to 14 days at temperatures above 70° F. Occasionally symptoms develop on the inoculated leaf, and rings may form along the inoculation scratches. This is often found after a series of progressive inoculations through successive plants, and is a point of difference from the "ringspot" mosaic of potato where local symptoms never occur. It often happens in needle inoculation of tobacco ringspot that some inoculations do not "take" on certain plants, about one in six failing to develop the disease. This seems to be due to some idiosyncrasy in the individual plant as conditions of inoculation are identical. The symptoms of ringspot in tobacco may develop in two ways, either on the inoculated leaf as is shown in Plate III, fig. 4, where necrotic rings form along the needle scratches, or, more usually, as a mottling on the youngest leaves of the plant, the rings developing later. In potato the "ringspot" mosaic always develops on the youngest leaves, never at the inoculation point.

(*b*) *By aphis.*

The source of infection in the first experiment was the White Burley plant illustrated in Plate I, fig. 2, which exhibited brilliant necrotic rings. This plant was colonised with the aphis *M. persicae* and, after 4 days, these were transferred to three White Burley and three Virginia plants. Nine days later the White Burleys showed the "spot mottle" disease which is typical of aphis infection of healthy tobacco plants from mosaic

potato. The Virginia plants remained healthy. In the second experiment White Burley plants, showing a very virulent form of necrotic mottling, which had been produced by progressive inoculations (see Plate III, fig. 3), were colonised with the aphis as before, and these were transferred later to healthy White Burley plants. In 12 days these White Burleys developed the mottling typical of aphis infection. It is of interest here to compare the results of a parallel series of needle inoculations from the same source, whereas the virulent form of necrosis, when transmitted by aphis, produced a mild mottling, the same when transmitted by needle produced a still more severe form of necrosis. Three points arise from these experiments: firstly they indicate that ringspot is an aphis-borne disease among tobacco plants, at least of the variety White Burley; secondly they emphasise the greater resistance of Virginia to aphis-borne ringspot; and thirdly they offer further evidence that the same virus, when aphis transmitted, produces different symptoms in the healthy plant from those produced by the virus when needle transmitted. Two possible causes may be suggested for this: either the virus has undergone some change in the body of the aphis, or else the different symptoms are induced by the different mode of inoculation to the plant.

Section 6.

Transmission of potato "ringspot" or intensified mosaic to healthy potato plants:

(*a*) *By needle.*

Seven series of experiments with needle inoculation of the intensified form of potato mosaic into healthy potatoes were carried out upon two varieties, Arran Victory and President, involving about twenty-five plants. The symptoms of the disease arising in the inoculated plants differed in no way from those produced by inoculation with tobacco ringspot on potato, the same brilliant mottling and necrotic spots being present. The disease developed in periods of 10 to 19 days, provided the temperatures were not above 80° F. The "ringspot" mosaic was just as easily passed by needle inoculation from potato to potato as from ringspot tobacco to potato.

(*b*) *By grafting.*

The "ringspot" mosaic was easily communicable from diseased to healthy potato plants by grafting. The symptoms appearing in about 9 days at temperatures below 80° F.

(*c*) *By aphis.*

Six series of experiments to induce the aphis *M. persicae* to transmit the "ringspot" mosaic from infected to healthy plants proved negative; both haulm and sprouted half tubers were colonised with the aphis without result. It occurred to the writer that the condition of the infected plant used as the source of infection for the aphis might be of importance. Consequently experiments were tried using as the source plants newly infected with "ringspot" mosaic and showing the symptoms very markedly. These experiments were negative also, there being no result whether the source was a diseased plant of long standing or one newly infected.

SECTION 7.

What is the effect on the virus of tobacco ringspot of progressive inoculations through successive generations of tobacco plants?

A series of progressive needle inoculations of ringspot was made in order to determine the changes, if any, induced in the virus by passage through succeeding generations of tobacco plants; three sets of experiments were performed. In the first experiment a start was made with juice from a mosaic Arran Victory potato plant, which produced concentric rings in Virginia tobacco. This ringspot was passed through five generations of tobacco plants, Virginia predominating, the times of development of the symptoms being 7 to 8 days between each set of plants. At the third set of plants, necrotic rings formed along the inoculation scratches (Plate III, fig. 4), but this did not always happen, and at the last series no perceptible increase in virulence of the virus could be detected. In the second experiment, starting with the same mosaic potato juice, the virus was passed through eight succeeding generations of tobacco plants, almost wholly of the variety White Burley. The seventh series of plants exhibited a most brilliant necrotic ringing (Plate I, fig. 3), unusual in the variety White Burley, which does not easily produce rings. In the eighth series of plants both varieties were used, and here the virus showed signs of loss of virulence and a tendency to return to the mottle and ringspot form of early infections. Furthermore, the brilliant rings did not persist throughout the life of the individual plant but, as new leaves were formed, the symptoms reverted to the characteristic spot necrosis of White Burley.

The third experiment gave somewhat different results, and was performed as follows: in the transmission experiments of potato mosaic virus to healthy tobacco, it was noticed that one series of Virginia plants

inoculated from a particular mosaic Arran Victory potato exhibited an unusually severe type of necrosis (Section 1, Exp. 4). One plant of this series was therefore selected for the third trial of successive inoculations, and juice from this plant was inoculated through a succeeding series of White Burley and Virginia plants. The first series, consisting of White Burley plants only, developed the spot necrosis type of disease in an accentuated form after 14 days. The second series of plants, White Burley and Virginia, developed in 10 and 12 days respectively an exceedingly severe type of necrosis of the veins. In the White Burleys the plants were almost killed, while the Virginias exhibited a very severe necrosis. The virus from one of these necrotic plants was then passed on to another series of White Burley and Virginia plants, which developed symptoms in 11 days. These plants showed a further increase in virulence of the virus and were practically destroyed. It is worthy of note that half-grown plants inoculated with this virus were not nearly so seriously affected as the young seedlings. That this severe disease was ringspot in some form was shown by the occurrence of isolated rings on the leaves of the Virginia before they were destroyed by the virulent necrosis. Plants infected with this virus developed new leaves which grew normally for some days until the necrotic lesions developed on them.

In this case, therefore, the plants did not grow away from the severe symptoms as had been the tendency hitherto, but succumbed to the disease. Some evidence is thus offered that continued passage of the ringspot virus through successive generations of tobacco plants does lead to an increase in the virulence of the virus, especially where the virus passes through a plant host which is favourable to it, such as the tobacco variety White Burley. As a general rule this increase in virulence is maintained in individual plants for a period only, the severe form of symptom, ring or necrosis, is exhibited only for a short time after inoculation; later new leaves appear which develop a less virulent form of symptom (Plate III, fig. 3). That this is not always the case is shown by the third experiment in this series where progressive inoculation increased the virulence of the disease until it killed the plants. It is possible that some additional factor of which the writer has no knowledge was present in this case.

In conclusion, the suggestion is made that passage of the ringspot virus through a succession of plants, favourable to its development, *does* produce an increase in virulence but usually only up to a point; when that point is reached the virus tends to return to and maintain its original level of intensity. Further experimentation is being carried out on this point.

SECTION 8.

What is the effect on the virus of potato "ringspot" or intensified mosaic of progressive inoculations through successive generations of potato plants?

A parallel series of progressive inoculations to those carried out in the tobacco was performed to determine if any increase in virulence resulted. This experiment was only carried as far as the fourth plant, but so far as it went there were no indications of increasing virulence. Each plant developed the typical mosaic mottling and necrotic lesions.

SECTION 9.

Inoculation of healthy tobacco plants with the virus of potato "ringspot" or intensified mosaic:

(*a*) *By needle.*

The virus of the intensified form of mosaic resulting in potatoes inoculated from ringspot tobacco, was returned again to healthy tobaccos. Six series of tobacco plants were inoculated from different potatoes showing the intensified mosaic. The symptoms developed in the tobacco plants in periods of 11 to 19 days. Sometimes in Virginia plants the typical rings were formed, but much more usually the first symptoms consisted of a type which might be called "clearing of the veins" (Plate II, fig. 5). This type of symptom seems especially characteristic of ringspot virus that has passed through potato. This clearing of the veins may persist for a longer or shorter time, but is generally a prelude to the development of the "mottling" manifestation of ringspot. From these experiments it would appear as if passage through potato has not materially altered the ringspot virus. It still shows a tendency to form rings upon Virginia tobacco and the mottling produced in both varieties does not differ very materially from that produced on many occasions by the virus when in the form of ordinary potato mosaic it was first transmitted to tobacco.

(*b*) *By aphis.*

Aphides (*M. persicae*) were colonised upon an Arran Victory potato affected with typical intensified or "ringspot" mosaic, later they were transferred to healthy White Burley tobacco plants. Symptoms of mottling developed on the tobacco in 12 days, this mottling did not differ in appearance from that produced by aphis inoculation with ordinary potato mosaic. This experiment shows that the intensified form of mosaic in potato is transmissible to tobacco by the aphis *M. persicae*.

SECTION 10.

Needle inoculation of healthy tobacco plants with the juice of known healthy Arran Victory potatoes:

Recent work in America has suggested the possibility of healthy potatoes carrying a virus which may be toxic to other plants, and Johnson(3) has found that inoculations with juice from apparently healthy potatoes produces a disease in tobacco. Four series of inoculations with the juice of healthy potato plants into healthy tobacco were carried out. The potatoes used in this experiment were part of a stock of known healthy Arran Victory grown for three years under insect-proof conditions. No symptoms of any kind resulted in the tobacco plants thus inoculated. The writer would suggest from this that the potatoes used by Johnson, although to all appearances healthy, must have been "carrying" mosaic, that is, infected with the disease but not showing the symptoms. Carriers are known of most if not all the other potato virus diseases, and the same may be true of mosaic.

SECTION 11.

Effect of temperature on the symptoms of ringspot:

(*a*) *In potato.*

There seems little doubt that temperature plays an important part in the development of this disease, both in tobacco and potato. All symptoms of the "ringspot" or intensified form of mosaic are suppressed in the potato above 80° F. (Plate V, fig. 2), and it is even difficult to infect a potato plant with the disease at or above that temperature. "Ringspot" mosaic flourishes best in the potato at about 60° to 65° F. In the glasshouse it was found that the symptoms appeared and disappeared with the fall and rise of temperature. As the details of transmission experiments of ringspot to potato will show, the times elapsing between inoculation of the potato and the appearance of the symptoms increases as the temperature rises, this being the exact reverse of what occurs in tobacco.

(*b*) *In tobacco.*

Below about 50° F. the tobacco plant will not flourish but remains in a stationary condition. In these circumstances, although it is possible for a plant to be infected, no symptoms of ringspot manifest themselves until the temperature rises above 65° F. The symptoms, however, of a tobacco plant once infected with ringspot do not disappear at low tem-

peratures. The disease in tobacco seems to show best between 75° to 80° F. Tobacco plants inoculated with potato mosaic on December 7th, did not manifest the true ringspot until January 20th, a total of 44 days at the low temperatures then prevailing. On the other hand, healthy tobaccos inoculated with potato mosaic on June 26th developed typical ringspot symptoms on July 8th, a total of 12 days, at a mean daily maximum temperature of 82° F. It may be seen at once from the details of the transmission experiments of potato mosaic to tobacco that the time elapsing between inoculation of the tobacco and appearance of the symptoms decreases, within limits, as the temperature rises, and the same is essentially true of the aphis transmission.

Filtration of the virus:

Through the kindness of Dr Henderson Smith, juice from a ringspot Virginia tobacco plant showing the typical double rings, and from an Arran Victory potato plant with "ringspot" mosaic was filtered in the following manner(2). The plants were pulped and passed first through muslin, then through tubes of sand and paper pulp, and finally through two Pasteur-Chamberland filter candles, L 1 and L 3 respectively. Some of the resulting fluid was inoculated three days later as follows: the juice from the ringspot Virginia tobacco was put into six Virginias, six White Burleys, and three healthy Arran Victory potato plants. The filtered juice from the "ringspot" Arran Victory potato was put into three Virginia tobacco, and three healthy Arran Victory potato plants. The results were as follows: the three potato plants inoculated from the ringspot Virginia developed typical "ringspot" mosaic in 11 days, while the healthy Virginias, inoculated with the same inoculum, developed the disease in 12 days. The three healthy potatoes inoculated with the filtered juice from the "ringspot" mosaic potato developed typical "ringspot" mosaic 16 days later, as did also two of the Virginia tobacco plants.

This seems conclusive evidence that the ringspot disease of tobacco is a filterable entity.

SECTION 12.

Inoculation of tobacco plants with virus combinations of which potato mosaic is a constituent part:

Two virus combinations only have so far been used in this series of experiments, *i.e.* streak and mosaic inoculated with the needle, and leaf-roll and mosaic inoculated by means of the aphis *M. persicae*.

The streak-mosaic combination was produced by grafting a mosaic Arran Victory with a streak-carrying Up-to-date plant. When the streak

symptoms were fully developed upon the Arran Victory, juice from this plant was inoculated by the needle into two series of White Burley tobacco. A mottling disease developed in 6 days and 14 days respectively. This mottling did not appear very different from that produced in the same kind of tobaccos by inoculation with potato mosaic only. Juice from these tobaccos was then inoculated into a series of healthy Arran Victory and President potato plants. In 9 days all the potato plants developed the typical intensified form of mosaic produced by inoculation with ringspot tobacco. There were no signs of streak in any of the potato plants, both varieties of which are exceedingly susceptible to this disease.

Leaf-roll and mosaic. This combination was produced by the infection of a mosaic Arran Victory with leaf-roll by means of the aphis *M. persicae* which the writer has shown to be an efficient carrier of this disease. Aphides (*M. persicae*) were colonised on this potato plant and later transferred to White Burley tobaccos. In these plants the typical aphis-mottle was produced, in no way differing from that associated with aphis inoculations from mosaic potato only. That the aphis in this case was carrying both viruses, and that the tobacco only received one has been shown by the writer in another communication, shortly to be published on the aphis transmission of leaf-roll.

From these preliminary experiments with virus combinations upon tobacco, some evidence is presented that potato mosaic only is associated with ringspot of tobacco and its various manifestations.

Section 13.

Needle inoculation of plants other than tobacco or potato with the virus of tobacco ringspot:

(*a*) *Solanaceae.*

Tomato plants developed a mottling on the leaves but the writer has not yet succeeded in producing rings.

Petunia also showed mottling with some slight distortion of the leaves.

Datura inoculated with ringspot White Burley which showed very necrotic rings (Plate II, fig. 2), developed first a mottling which later turned into large necrotic lesions (Plate IV, fig. 1). True rings have not yet been produced in Datura, though a slight tendency to a ring-like form has been observed.

Solanum nigrum gave no symptoms when inoculated with tobacco ringspot.

(*b*) *Other plants.*

Negative results have been obtained by inoculation of ringspot virus into (*a*) Spinach, (*b*) Cabbage.

6. DETAILS OF EXPERIMENTS.

Section 1 (*a*). *Needle inoculation of tobacco with potato mosaic.*

No. of exp.	No. of plants inoculated	Date* of inoculation	Appearance of first symptoms	Incubation period days	Mean daily maximum temperature ° F.	No. of plants infected	Symptoms
1	3 White Burley 3 Virginia	Dec. 7	Jan. 10	34	57	3 W.B. 2 V.	Mottling followed by double rings on Virginia and spot necrosis on White Burley.
2	3 White Burley 3 Virginia	Feb. 23	Mar. 15	21	72	3 W.B. 3 V.	Rings on Virginia; spot necrosis on White Burley.
3	6 White Burley	Mar. 3	Mar. 20	17	74	4 W.B.	Spot necrosis.
4	6 Virginia	Mar. 8	Mar. 22	14	75	6 V.	Rings in most cases; some mottling. Severe necrosis in one plant.
5	3 White Burley 3 Virginia	June 26	July 8	12	82	3 W.B. 3 V.	Spot necrosis on White Burley concentric rings and nebulous rings on Virginia.
6	3 White Burley	June 27	July 10	13	82	3 W.B.	Spot necrosis. Half spot, half ring in one plant.

Section 1 (*b*). *Aphis* (M. persicae) *inoculation of tobacco with potato mosaic.*

1	3 White Burley 3 Virginia	Dec. 7	Jan. 20	44	62	3 W.B.	Characteristic "spot mottle" on White Burley; Virginia healthy.
2	6 White Burley 6 Virginia	Feb. 22	Mar. 23	30	73	5 W.B. 2 V.	Typical mottle later turning to spot necrosis.
3	3 White Burley	Apr. 24	May 11	17	82	3 W.B.	Typical aphis mottle.
4	3 White Burley 3 Virginia	June 15	June 30	15	87	3 W.B.	Typical aphis mottle.
5	3 White Burley	June 10	June 25	15	90	2 W.B.	Typical aphis mottle.
6	6 White Burley	June 28	July 15	17	84	5 W.B.	Typical mottle with nebulous "watermark" type of ring on one plant.

* In Section I (*b*) date of inoculation = date of colonisation of plant with the aphis.

Section 2 (a). Needle inoculation of potato with tobacco ringspot.

No. of exp.	No. of plants inoculated	Date of inoculation	Appearance of first symptoms	Incubation period days	Mean daily maximum temperature ° F.	No. of plants infected	Remarks
1	6 Arran Victory	Feb. 21	Feb. 27 to Mar. 3	6–11	68	6	—
2	3 Arran Victory	Mar. 5	Mar. 12	7	71	3	—
3	2 Arran Victory, 2 President	Mar. 7	Mar. 18 and Mar. 21	11–14*	69	4	—
4	3 Arran Victory (inoculated with filtered virus)	Apr. 4	Apr. 16	12	77	3	—
5	6 President	Apr. 13	Apr. 26	13	76	6	—
6	6 President	May 2	May 11	9	75	6	—
7	3 President	May 7	May 16	9	77	3	—
8	3 President	June 4	June 25 and July 2	21 and 28	85 and 86	3	—
9	3 President	May 15	May 26	11	79	3	—
10	3 President	June 2	June 20	18	84	3	—
11	3 Arran Victory	June 2	June 18	16	80	3	—
12	3 Arran Victory	June 11	June 27	16	80	3	—
13	3 Arran Victory	June 14	June 30	16	80	3	—
14	4 President	June 16	June 30	14	80	4	—
15	3 half tubers Arran Victory (inoculated in sprouts)	May 26	June 25	40	86	3	—

* 14 days = older Arran Victory; 11 days = young President.

Section 2 (b). Inoculation of potato with tobacco ringspot by aphis.

1	3 President	Mar. 13	Mar. 27	14	68	1	Young seedlings used.
2	5 President (2 of above exp. re-infected)	Mar. 16	—	—	68	Negative	—
3	3 President	Apr. 16	May 10	24	75	1	Seedling plants used.
4	3 President	Apr. 16	Apr. 30	14	72	1	Slight mottling only.
5	3 President	Apr. 20	—	—	75	Negative	In this experiment a newly infected plant was used as source of infection.
6	24 sprouted Arran Victory half tubers with half tuber controls	Apr. 22	—	—	75	Negative	—
7	3 Arran Victory	May 3	—	—	75	Negative	—

In experiment No. 7 three young Arran Victory plants were colonised with aphis from a tobacco plant which had itself been infected with the mottle disease by aphis from mosaic potato. Juice from this tobacco plant when inoculated by needle into healthy potato produced the typical "ringspot" or intensified mosaic. The aphis, however, failed to carry the disease back to potato, although it brought it readily enough to the tobacco.

Section 3. See page 14.

Section 4. *Needle inoculation into healthy potato of the aphis-produced mottle in tobacco.*

No. of exp.	No. of plants inoculated	Date of inoculation	Appearance of first symptoms	Incubation period days	Mean daily maximum temperature ° F.	No. of plants infected	Remarks
1	3 President	Apr. 12	—	—	—	—	These three plants remained apparently healthy.
2	4 President	May 14	June 4	21	82	4	The inoculum used in this experiment was the same as in No. 1, but was passed through an additional generation of tobacco.
3	4 President	May 14	May 26	12	76	4	—
4	3 Arran Victory	May 31	June 7	8	80	3	Symptoms were masked by temperature on June 7 and disappeared until July 2.
5	3 President	June 20	July 10	20	80	3	—

Section 5 (*a*). *Needle transmission of tobacco ringspot to tobacco.*

No. of exp.	No. of plants inoculated	Date of inoculation	Appearance of first symptoms	Incubation period days	Mean daily maximum temperature ° F.	No. of plants infected	Remarks
1	3 White Burley	Jan. 30	Feb. 14	15	63	3	—
2	3 White Burley	Feb. 15	Feb. 27	12	78	3	—
3	6 Virginias	Feb. 15	Feb. 22	7	78	5	—
4	3 Virginias	June 21	July 1	10	86	2	—
5	3 Virginias	Feb. 17	Mar. 3	15	64	3	—
6	3 White Burley	Feb. 16	Mar. 3	14	64	3	—

Section 5 (*b*). *Aphis transmission of tobacco ringspot to tobacco.*

1	3 White Burley 3 Virginia	July 28	Aug. 7	10	83	3 W.B. Virginia is apparently negative	The aphides were colonised on tobacco with pronounced "rings," but the disease produced in the new tobaccos, was the typical "spot mottle."
2	4 White Burley	Aug. 15	Aug. 27	12	80	4 W.B.	—

In experiment 2 the aphis were colonised on White Burley tobacco which was affected with the very severe type of necrosis; the four infected plants, however, showed only the typical aphis mottle.

Section 6 (a). Needle transmission of potato "ringspot" or intensified mosaic to healthy potato plants.

No. of exp.	No. of plants inoculated	Date of inoculation	Appearance of first symptoms	Incubation period days	Mean daily maximum temperature ° F.	No. of plants infected	Remarks
1	3 Arran Victory 3 President	Mar. 30	Apr. 10	11	78	6	Source of infection was a "ringspot" Arran Victory.
2	3 half-grown Arran Victory	Mar. 30	Apr. 16 to Apr. 18	17–19	75	6	Illustrates the shorter incubation period in younger plants.
	3 very young Arran Victory		Apr. 11	12	78		
3	3 Arran Victory	Apr. 2	Apr. 18	16	78	3	Inoculum used was filtered (see Section 11).
4	3 President (seedlings)	May 25	June 4	10	78	3	—
5	3 President (seedlings)	June 6	July 2	34	85	3	Symptoms masked by temperature.
6	1 Arran Victory 1 President	June 25	July 10	15	78	2	—

Section 6 (b). Transmission by grafting of potato "ringspot" mosaic to healthy potato.

1	Arran Victory grafted with diseased scion of Arran Victory	May 26	June 10	14	78	1	—
2	Arran Victory grafted with diseased President	May 26	July 1	35	82	1	Symptoms masked by temperature.

Section 6 (c). Aphis transmission of potato "ringspot" mosaic to healthy potato.

Six series of experiments on both haulm and sprouting half tuber. All these were negative.

Section 7. What is the effect, on the virus of tobacco ringspot, of progressive inoculations through successive generations of tobacco plants?

No. of exp.	Generations of tobacco	Date of inoculation	Appearance of first symptoms	Incubation period days	Remarks
1	(*a*) Virginia	Feb. 15	Feb. 22	7	In (*d*) typical necrotic rings formed along the inoculation scratches (Plate III, fig. 4).
	(*b*) White Burley	Feb. 16	Feb. 24	8	
	(*c*) Virginia	Mar. 23	Apr. 1	8	
	(*d*) Virginia	May 2	May 10	8	
	(*e*) Virginia	May 23	May 31	8	Exp. No. 1 finished at (*e*); there was no perceptible increase in virulence.
2	(*a*) Virginia	Feb. 1	Feb. 8	7	Both Exps. 1 and 2 started with the same virus. In Exp. 2, at the 5th generation (*e*), very brilliant necrotic rings developed (Plate I, fig. 2).
	(*b*) Virginia	Feb. 15	Feb. 22	8	
	(*c*) White Burley	Feb. 16	Feb. 24	8	
	(*d*) White Burley	Mar. 23	Apr. 2	9	
	(*e*) White Burley	June 25	July 4	9	
	(*f*) White Burley	July 30	Aug. 10	11	In (*f*) the symptoms have reverted to characteristic spot necrosis.
3	(*a*) Virginia	Mar. 8	Mar. 24	16	The source of inoculation in Exp. 3 was a Virginia plant showing unusually necrotic spotting, once from mosaic potato.
	(*b*) White Burley	June 4	June 27	23	Necrosis increased in (*b*).
	(*c*) White Burley	July 26	Aug. 5	10	All plants show a very severe form of necrosis (see Plate III, fig. 3).
	Virginia	July 26	Aug. 7	12	
	(*d*) White Burley	Aug. 9	Aug. 20	11	Virulence still further increased. Plants practically killed.

Section 8. See page 19.

Section 9 (a). Needle inoculation of healthy tobacco plants with the virus of potato "ringspot" or intensified mosaic.

No. of exp.	No. of plants inoculated	Date of inoculation	Appearance of first symptoms	Incubation period days	No. of plants infected	Remarks
1	6 Virginia	Mar. 7	Mar. 21	14	5	Some rings, and mottling.
2	6 Virginia	Mar. 16	Mar. 23	17	6	Majority of plants show "clearing of the veins." Some rings.
3	3 Virginia	Mar. 30	Apr. 10	11	3	Symptoms typical of ringspot.
4	3 Virginia	Apr. 2	Apr. 21	19	3	Inoculum used was filtered juice. Symptoms typical rings but faint. Virus appeared slightly attenuated by filtration.
5	3 White Burley	June 25	July 7	12	3	"Clearing of the veins" followed by mottling. Symptoms died out in one case.
6	3 White Burley, 3 Virginia	July 30	Aug. 8	9	3 W.B., 1 Virginia	"Clearing of the veins."

Section 9 (b). Aphis inoculation of healthy tobacco plants with the virus of potato "ringspot" or intensified mosaic.

1	3 White Burley	Aug. 19	Aug. 31	12	3	A mottling disease produced which did not differ in appearance from that produced by aphis inoculation with ordinary potato mosaic.

7. DISCUSSION.

The outstanding experience of the writer in his work with the insect transmission of potato mosaic has been the difficulty of obtaining an insect which would carry the virus with any degree of regularity. This difficulty does not seem to be shared with other potato virus workers on the continent and in the United States (Elze (1), Schultz and Folsom (6)). The aphis *Myzus persicae*, so largely used in this work, is the insect to which the writer has paid greatest attention in his virus studies because it is the one which has given nearly all the positive results obtained. Not only is it the chief, if not the sole, vector of potato leaf-roll in this country, but it is concerned, practically all over the world, with the dissemination of plant viruses of many kinds. This apparent affinity for virus diseases, emphasised by its omnivorous habits and world-wide distribution, renders *Myzus persicae* an insect of the gravest importance.

The non-success of the insect transmission of potato mosaic led to the experimentation with the tobacco plant described in this paper. The utilisation of the tobacco as an indicator has demonstrated one important fact, *i.e.* that the aphis *M. persicae* does actually pick up the virus when it feeds upon a mosaic potato plant. The demonstration of this fact renders the reasons for its failure to infect the potato, under the writer's experimental conditions, difficult to fathom. As the foregoing experiments indicate, the aphis infects the tobacco from the mosaic potato with great ease and will also carry the virus on from tobacco to tobacco, but immediately the infective aphis is brought into contact with the potato, the positive infections cease. Inoculation of tobacco with the virus of a potato mosaic has given rise to several further points of importance. It has demonstrated that a potato mosaic and tobacco "ringspot" are apparently caused by the same virus; this immediately suggests the potato fields as a source of tobacco ringspot in America, with the aphis *M. persicae* as the probable connecting link. That the same virus in another plant host should react in so different and striking a manner is of considerable interest. Moreover, the symptoms of the disease in tobacco, when in the ringspot form, are particularly striking. What is it that causes a disease, the usual symptoms of which in one plant are a mild mottling, to develop in another plant host the peculiar double concentric rings shown in Plate I, figs. 1 and 2, and what is the significance of rings? These rings are symmetrical and very clearly cut, giving the appearance of being stamped on artificially. It is probable that the virus of potato mosaic undergoes some change in the tissues of the tobacco

plant. This change does not appear to be of a fundamental nature, because ringspot returned to potato reappears in a mosaic form. It is true that the symptoms are intensified and the infective power enhanced, nevertheless it is still a disorder of the mosaic type. There are other points of difference in the behaviour of the virus in the two plant hosts—in tobacco the symptoms show best at 80° F., while in potato the symptoms are masked at that temperature and positive inoculation is difficult if not impossible. As the potato plant grows well at 65° F., and the tobacco at 80° F., and as it is at these temperatures that the symptoms show best in the respective plants, optimum conditions for the plant host are apparently optimum conditions for the virus. In tobacco local symptoms often appear at the point of inoculation, but never in potato where first symptoms always develop on the young leaves. A parallel case of one virus being concerned in two different diseases is found in the work of Kunkel(4) who shows that Aster Yellows is caused by the same virus as that producing White Heart of lettuce.

A further point of interest lies in the results obtained by respective inoculations of tobacco by needle and aphis. Although the same mosaic Arran Victory potato was used as the source of infection in both cases the resulting symptoms were entirely different, each one characteristic of its method of inoculation. The needle inoculation produces the rings or typical spot necrosis, while aphis infection produces symptoms of a marked type consisting of a yellowish spotting accompanied by a mottling of darker green[1]. Also, when the severe form of disease illustrated in Plate III, fig. 3, was inoculated into fresh plants by means of the needle, death resulted in the inoculated plant, but when transmitted by the aphis the typical aphis mottle only resulted. It may be suggested that the virus has undergone a change in the body of the aphis, but if so the change is presumably not great, as, when returned to healthy potato by needle, the disease produced is symptomatically identical with that produced by true ringspot. It may be that this disparity in symptoms arising between aphis and needle inoculation of tobacco is due to the difference in the mode of inoculation. The writer has shown(7) that *M. persicae* consistently taps the phloem of its plant host, so that presumably the virus is introduced to the phloem of the tobacco plant by the aphis with greater consistency than by a needle scratch on the leaf blade. This difference may be sufficient to account for the divergent symptoms. Comparative inoculations of the two varieties of tobacco, White Burley

[1] The lines of darker green shown in Plate III, fig. 1, are very characteristic of aphis-borne infection of tobacco plants.

and Virginia, have demonstrated that there exists between them a considerable difference of susceptibility to the ringspot virus. This difference in susceptibility is particularly strong in the case of aphis-borne infections. It is as easy to infect White Burley tobacco with the spot mottle disease by means of the aphis as it is difficult to infect Virginia tobacco with the same disease by means of the aphis. This varietal susceptibility is also shown though to a less extent in needle inoculations. Were it not for the fact that both the varieties of potato used, *i.e.* Arran Victory and President, are exceedingly susceptible to the disease, difference in varietal susceptibility to mosaic might explain the writer's non-success with aphis transmission of this disease to potato.

It is a commonplace of plant viruses that active growth and movement in the host is an essential for infection and subsequent development of the virus. This is emphasised in the present studies where young potato plants inoculated with the virus of ringspot developed symptoms in 12 days, as compared with 17 to 19 days in older plants under similar conditions. Further, tobacco plants in a pot-bound, and consequently stationary, condition were very difficult to infect with ringspot. Symptoms in tobacco plants already infected, tended to disappear when the plant became pot-bound and to reappear when the plant re-started into active growth. It has been shown that ringspot in tobacco, and its counterpart in potato are filterable entities; in this the writer's results differ from those of Priode(5), who found that the ringspot disease of tobacco would not pass a grade "N" Berkfeldt filter.

It is evident that much work yet remains to be done both concerning insect transmission of potato mosaic and the affinities of this disease with tobacco ringspot. Although at the moment some of the results obtained may appear incompatible, it is hoped that, as knowledge of a difficult subject grows, these apparent anomalies may disappear.

8. SUMMARY.

(1) Some positive evidence of the transmission of potato mosaic by the aphis *Myzus persicae* Sulz. is given.

(2) Attempts to induce a number of other potato-feeding insects to transmit the disease proved negative.

(3) It has not been found possible to infect potato plants with mosaic by means of inoculation with the body juices, or salivary glands, of insects which have been bred upon mosaic potato plants.

(4) The virus of a potato mosaic inoculated into healthy tobacco plants by means of the needle produces an infectious disease—ringspot—

of which the most characteristic symptom is the formation of necrotic concentric rings with a central spot. The symptoms of this disease differ in the two varieties of tobacco used—White Burley and Virginia—and the susceptibility of the former is greater.

(5) The virus of a potato mosaic transmitted to healthy tobacco by means of the aphis *M. persicae* produces a characteristic spot and mottle disease which is considered to be substantially the same disease as the needle-produced ringspot.

(6) Ringspot in its various manifestations, when inoculated back to healthy potato by needle or aphis, reproduces in the potato the original mosaic with the symptoms intensified, and the infective power greatly enhanced. Although the aphis readily carries the virus of potato mosaic to tobacco, it transmits the resulting disease back to potato only with very great difficulty.

(7) This intensified mosaic can be spread from potato to potato by needle scratch with the greatest ease, but not by the aphis *Myzus persicae* Sulz.

(8) The spot and mottle disease produced in tobacco by aphis from mosaic potato, when inoculated into healthy potato plants by the needle, produces the same intensified form of mosaic as does the needle-induced ringspot in tobacco, when returned to healthy potato.

(9) Tobacco ringspot can be spread from tobacco to tobacco by needle or aphis. When transmitted by aphis the symptoms differ from those produced by the needle.

(10) It is proved that the aphis *Myzus persicae* picks up the virus from mosaic potato with great regularity, but has so far usually failed under the writer's experimental conditions to infect healthy potato plants with the disease.

(11) The virus of tobacco ringspot is shown to increase in virulence by progressive inoculation through successive generations of tobacco plants. The increase is greatest after passage through a plant which is highly favourable to the development of the virus, such as the susceptible variety of tobacco, White Burley. This increase in virulence in some cases reaches only to a certain point, after that it tends to revert to its original intensity, but in others the virulence reached an intensity sufficient to kill the plant. As a rule increased virulence of the virus in a given plant does not persist throughout the life of that plant.

(12) Temperatures above 80° F. mask the symptoms of the intensified mosaic in potato, but not of ringspot in tobacco. The respective symptoms show best in potato at 60° to 65° F. and in tobacco at 80° F. Needle

inoculation on tobacco often produces local symptoms at the point of inoculation, but never on the potato where first symptoms appear on the young leaves.

(13) Juice from a potato plant infected with the intensified mosaic, and from a ringspot tobacco plant, was filtered through two Pasteur-Chamberland filter candles, L 1 and L 3. The resulting filtrate originating from each plant infected healthy potato and tobacco plants with their respective diseases. This shows that the ringspot virus, and its counterpart in potato, are filter-passing entities.

(14) Juice from known healthy potato plants when inoculated into healthy tobacco plants produced no symptoms.

(15) The virus of tobacco ringspot was inoculated into various plants with the following results:

(*a*) Tomato. Mottling on the leaves, rings not yet produced.
(*b*) *Petunia.* Mottling on the leaves with some distortion.
(*c*) *Datura* sp. Mottling accompanied by large necrotic areas.
(*d*) *Solanum nigrum.* No symptoms.
(*e*) Spinach. No symptoms.
(*f*) Cabbage. No symptoms.
Only two plants each inoculated in (*d*), (*e*), (*f*).

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Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 1.

Fig. 2.

Fig. 4.

Fig. 3.

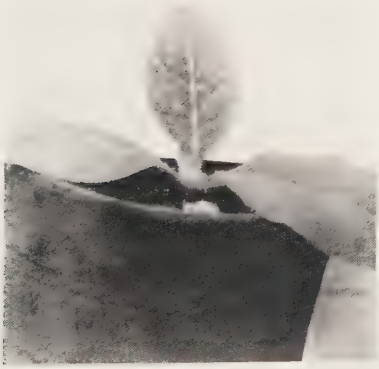

Fig. 5.

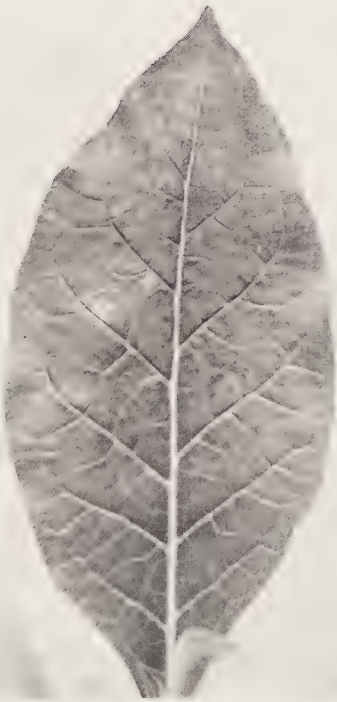

Fig. 6.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 1.

Fig. 2.

SMITH.—Studies on Potato Virus Diseases (pp. 1-33).

EXPLANATION OF PLATES I—V.

PLATE I.

Fig. 1. Typical ringspot symptoms on Virginia tobacco. These symptoms are the result of a direct inoculation from mosaic Arran Victory potato. Note the double rings in the lower right-hand corner. Average diameter of rings about 5 mm.

Fig. 2. Rings produced in White Burley tobacco, after passing the virus through five successive generations of tobacco. Size of leaf, 9 in., rings varied in diameter from 5 to 8 mm.

Fig. 3. Hieroglyphic type of marking produced in White Burley, usually after a number of progressive inoculations.

Fig. 4. Nebulous or "watermark" type of ring. This occurs in both varieties, and is the only type of ring yet produced by aphis inoculation from mosaic potato.

PLATE II.

Fig. 1. Young White Burley tobacco plant infected with ringspot, the rings are rapidly turning into the spot necrosis characteristic of the disease in this variety.

Fig. 2. Older White Burley plant, the leaves may be seen covered with spots and blotches, some of which still retain a ring-like form.

Fig. 3. Spot necrosis symptoms developing in young White Burley.

Fig. 4. Young White Burley inoculated with the same virus as in Fig. 3. Here the symptoms are tending towards ring formation.

Fig. 5. "Clearing of the Veins." A common early symptom of ringspot in both varieties of tobacco.

Fig. 6. Spot necrosis as it often appears in an old White Burley plant.

PLATE III.

Fig. 1. Leaf of White Burley plant showing symptoms produced by aphis inoculation from mosaic potato. At the time of photographing, this plant had been infected for some weeks. The typical spotting produced by the aphis may be seen at the apex of the leaf. Length of leaf, 7 in.

Fig. 2. Leaf of mature White Burley plant inoculated by needle from mosaic potato, for comparison with Fig. 2. Note the same picking out of the veins with darker green is found in both.

Fig. 3. Young Virginia plant inoculated with the virus which has been passed through a number of White Burley plants. Note the severe necrosis in two leaves. The new leaves show signs of growing away from the severe form of the disease, these leaves later developed necrosis less severe than in the older leaves.

Fig. 4. Rings forming along the inoculation scratches on a leaf of Virginia.

PLATE IV.

Fig. 1. Leaf of *Datura* plant inoculated with tobacco ringspot. Note the very severe necrotic lesions developing on the leaf. Size of leaf, $3\frac{1}{2}$ in.

Fig. 2. One of the few successful transmissions to potato by means of the aphis *M. persicae*. "Ringspot" or intensified mosaic in a healthy President potato plant produced by aphis from ringspot Virginia tobacco.

Fig. 3. "Ringspot" or intensified mosaic on President produced by needle inoculation from ringspot tobacco. The light and dark areas represent the characteristic mottling of this disease.

Fig. 4. "Ringspot" or intensified mosaic in Arran Victory produced by needle inoculation from ringspot tobacco. Photographed by transmitted light.

PLATE V.

Fig. 1. Leaf of Arran Victory potato, showing the large and small necrotic lesions occurring in "ringspot" mosaic. The small lesions appear in the photograph as numbers of black specks along the outer uppermost leaf margin.

Fig. 2. Stem on the left cut from potato plant showing the intensified form of mosaic, that on the right masked by temperature.

Photographs by C. W. Williamson.

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ON TWO CASES OF RECOVERY FROM A MOSAIC DISEASE OF TOMATO PLANTS, *LYCOPERSICUM ESCULENTUM*

By LEN VERWOERD, M.Sc. Agric. (Stell.).

(*Department of Phytopathology, College of Agriculture, University of Stellenbosch, Stellenbosch, C.P., Union of South Africa.*)

1. Introduction.

During recent years much has been added to our knowledge concerning the mosaic diseases which affect plants belonging to different families and orders. Although many obscure facts have found elucidation, there are still many perplexing questions which remain unsolved.

Most workers on mosaic diseases are agreed that if a plant has once become infected with mosaic there exists little hope for true recovery. Reports of recovery from mosaic diseases are naturally sceptically viewed by critical students of this problem. If such recovery is eventually proved, then it would appear to be of an exceedingly rare occurrence. The disappearance of mosaic symptoms have been observed by various workers. Allard (1) noticed that plants of *Nicotiana glauca* soon lost their mottled appearance, but he demonstrated the presence of the infective principle in such plants and found it highly virulent. This observation was later confirmed by Dickson (6) in his experiments on mosaic. Melhus (10) records that the eggplant, *Solanum melongena*, lost its mosaic characters after the plants had passed the seedling stage. Cases have been reported (3, 9, 11) of plants, which had previously shown mosaic infection, producing new shoots free from this disease. It is also known that *Physalis alkekengi* (11) and *Physalis francheti* (7) never show symptoms of mosaic mottling, though they may contain the active principle which causes mosaic symptoms when injected into tobacco plants.

None of the cases above referred to can be considered as indicating true recovery from mosaic. As far as I am aware, only one case (4) has thus far been reported which would tend to indicate the possibility of recovery from mosaic. This record is, however, based on negative results in only one inoculation trial.

The disappearance of symptoms of mosaic disease, which Lodewyks (8), Chapman (5), and Dickson (6) reports to take place when mosaic plants are

grown in blue light, was subsequently shown by Dickson (6) to be one of masking and not true recovery. This he demonstrated by infecting healthy plants which had been exposed to blue light and also by removing some of these plants to sunlight when they again developed mosaic symptoms.

2. EXPERIMENTAL.

During an epidemic spell of a mosaic disease of tomatoes in the months of December 1927 to February 1928 the writer had many thousand tomato plants under continual observation on the experimental farms of the University.

Three hundred tomato plants of the varieties Bonnie Best, Livingstone's Coreless and Norduke Wilt Resistant, which were infected with mosaic, were cut back at the time of flowering. A large percentage of these failed to produce new shoots. It is probable that the majority of the plants which did not again sprout were those most severely affected. A number of these plants which failed to sprout were dug up and their root systems examined. In all these cases the root development was of a normal character. In all cases where shoots developed the mosaic condition was reproduced in the new growth. The results of this experiment are recorded in Table I.

Table I.

	No. of plants				
Variety	With distinct mosaic symptoms	Cut back	De-veloping mosaic shoots	Not de-veloping mosaic shoots	Not de-veloping shoots
Bonnie Best	100	100	76	Nil	24
Livingstone's Coreless	100	100	60	Nil	40
Norduke Wilt Resistant	100	100	83	Nil	17
	300	300	219	Nil	81

Fifty tomato plants which showed no symptoms of mosaic and which were standing amongst those recorded in Table I were also cut back. These plants were not isolated and were liable to mosaic from the diseased plants by natural means. With three exceptions these plants developed healthy shoots showing no visible symptoms to indicate the presence of mosaic disease. The new growth of the three plants, which developed mosaic symptoms, could possibly have become infected by natural means from the diseased plants amongst which they stood. The results of this experiment are recorded in Table II.

Table II.

Variety	No. of plants: Apparently mosaic free	Cut back	Developing mosaic shoots	Not developing mosaic shoots	Not developing shoots
Bonnie Best	25	25	1	22	2
Norduke Wilt Resistant	25	25	2	23	0
	50	50	3	45*	2

* Thirty of the 45 plants which did not develop mosaic were subsequently used as a source for inoculum to test whether the disease was not perhaps latent or masked in these plants.

Allard (1) found that the virus of mosaic disease may be present in a highly virulent condition and yet not produce visible symptoms of mosaic on the host. This fact was subsequently confirmed by Dickson (6).

With the object of testing whether this was not perhaps also the case with the plants mentioned in Table II as being free from mosaic, the juice of thirty of these plants was extracted separately and each was injected into ten tomato plants raised under controlled conditions. Twelve weeks after inoculation the plants were growing vigorously and showed no signs of the disease on either foliage or fruit. At the same time mosaic-free tomato plants were inoculated by the same method with juice from diseased plants and these developed mosaic disease. Whilst visiting some tomato fields at Kleinvlei, Clanwilliam, in December 1927 a number of cuttings showing mosaic symptoms were taken from a badly infected plant and planted at Stellenbosch. An equal number of cuttings, taken from a mosaic-free plant, were planted out at the same time. Table III shows the result of this experiment and, as indicated there, the plants raised from two of the mosaic diseased cuttings showed no symptoms of mosaic.

Table III.

Date of planting	No. of cuttings planted	Mosaic or mosaic-free	No. of cuttings rooting	Subsequent growth: Mosaic	Subsequent growth: Mosaic-free	Date of final observation
Dec. 1927	24	Mosaic	6	4	2	10. iv. 28
	24	Mosaic-free	20	0	20	

In order to determine whether the infective principle was not perhaps latent or masked in the two healthy plants raised from diseased cuttings, the following infection experiments were made.

Twenty-five tomato plants (*vide* Series A, Table IV) were inoculated by means of the hypodermic syringe method with juice from the two

healthy and apparent mosaic-free plants mentioned in Table III. When three weeks had elapsed and no mosaic symptoms developed on the inoculated plants, two further series of twenty tomato plants were inoculated. The plants in the one series were inoculated by puncturing the host tissue through drops of juice (*vide* Series B, Table IV) and twenty tomato plants (*vide* Series C, Table V) by inserting small fragments of host tissue. The results of these inoculation experiments are given in Table IV.

Table IV.

Series	Date of inoculation	No. of plants	Variety	Method	Source of inoculum	Reaction	Remarks	Final date of observation
A	30. i. 28	25	Bonnie Best	Hypodermic syringe	The apparent mosaic-free plants	–	—	—
		25	Bonnie Best	Hypodermic syringe	Mosaic plant	+	Control	—
B	21. ii. 28	20	Bonnie Best	Punctures through drops of juice	The apparent mosaic-free plants	–	—	15. iv. 28
		20	Bonnie Best	Punctures through drops of juice	Mosaic plant	+	Control	—
C	21. ii. 28	20	Bonnie Best	Insertion of fragments of tissue	The apparent mosaic-free plants	–	—	—
		20	Bonnie Best	Insertion of fragments of tissue	Mosaic plant	+	Control	—

As may be inferred from the above table, no plants showed any symptoms of mosaic. The two mosaic-free plants raised from diseased cuttings eventually produced normal healthy fruit, free from mosaic.

In the course of the above experiments it appeared desirable to plant cuttings from the two mosaic-free plants raised from diseased cuttings, in order to ascertain whether the plants raised from them would remain free from mosaic. The season was by this time already well advanced, but nevertheless ten cuttings were planted from one of these plants. Only four of these cuttings rooted and the plants raised did not grow as vigorously as those planted from cuttings earlier in the season. Five weeks after planting the cuttings, the plants which developed showed no evidence of mosaic. Three tomato plants were inoculated with juice from these plants but without developing any signs of mosaic. From the experimental evidence cited, the author is led to conclude that the two mosaic-free tomato plants raised from diseased cuttings represent true recoveries from this disease.

3. Technique.

It seems desirable to give a brief account of the methods used in the experiments recorded.

Material. The tomato plants used in testing the transmissibility of the virus or infective principle were grown away from the pot-house where the inoculation experiments were carried out. The plants were raised from carefully selected seed in tins and the necessary precautions taken to exclude possible insect vectors. As soon as the young plants could be transplanted they were potted out in 6-inch pots. From amongst these plants vigorous ones, 5 to 10 in. high, were selected for inoculation experiments. This type of plant was selected because in some preliminary work on the interspecific transmission of mosaic diseases in Solanaceous plants such plants gave better results.

Inoculum. Except where otherwise stated, the juice of plants which served as inoculation material was obtained by macerating in a mortar the leaves and tips of young shoots of the plants. In this way a pulpy mass was obtained, which was diluted with a little distilled water and then filtered through filter-paper.

To obtain inoculation material containing the active principle of mosaic, plants were selected which showed well-developed symptoms of this disease.

Inoculation. Experiments in which plants were injected with an inoculum obtained from mosaic-free plants were controlled by injecting the juice of mosaic diseased plants into the same number of plants and *vice versa*.

In inoculating the plants, great care was taken to touch the plants as little as possible. Before introducing the inoculum the hands were thoroughly washed with soap and water. In each case, the plant to be inoculated was held between the thumb and index finger by means of a bit of cotton-wool, which was renewed for each plant. The juice was injected into the stem 2 in. above soil level and also at the base of a young leaf at the tip of the plant.

For injection a hypodermic syringe was generally used. In the case of the experiments mentioned in Table IV inoculations were also made: (*a*) by puncturing the host with a sterile needle through drops of juice obtained either from plants definitely known to be free from mosaic or from the two plants raised from mosaic-infected cuttings and which showed no visible sign of mosaic; (*b*) by inserting fragments of tissue obtained either from plants definitely known to be free from mosaic or

from the two plants raised from mosaic-infected cuttings and which showed no visible sign of mosaic, into the host by means of a curved arrowhead needle.

4. SUMMARY.

Experiments are recorded which indicate that from a number of tomato plants raised from mosaic-diseased cuttings two plants were free from mosaic disease.

These two plants showed no symptoms of mosaic disease and neither was the active principle of the disease present in the juice obtained from these plants.

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STUDIES OF WOOD-DESTROYING FUNGI

I. *POLYPORUS HISPIDUS* (FRIES)

By F. J. NUTMAN, A.R.C.S., B.Sc.

(*Department of Scientific and Industrial Research Forest Products Research Laboratory.*)

(With Plates VI–VIII and 2 Text-figures.)

CONTENTS.

Introduction.

Polyporus hispidus Fr. is one of the chief fungi attacking ash in this country. Rea (15) lists it as "common" and it is certainly of quite frequent occurrence, especially as it is one of those fungi which remain comparatively unnoticed until a search is made. This is due to the fact that the fruit body grows rather high up on the tree, and is of somewhat ephemeral nature.

It occurs generally on ash and apple. Rea gives walnut as an additional host, and I have frequently found small fructifications on elm, which is given as a host by Schroeter. Other hosts are mulberry (Prilleaux (14)), plane (Schroeter), and oak (Murrill (13)).

It is interesting to note that in America *P. hispidus* occurs on the Black Ash (*Fraxinus nigra*) almost exclusively, and where mixed stands of *F. nigra* and *F. excelsior* occur it attacks the former, never being found

on white ash, any decay of the latter being caused by *Fomes fraxinophilus* exclusively. In England *P. hispidus* occurs on both black and white ash, and I have collected sporophores from both. Owing, however, to the greater abundance of white ash, the fungus is chiefly important as causing a decay of this tree.

The decay produced in white ash is very similar to that described by Baxter (1), the decayed wood being conspicuously lighter in colour and softer than sound wood, and cracking along the growth rings when dried. In nearly all cases examined, the source of infection was the stub of a dead branch, the rot spreading up and down the tree for a considerable distance. The sporophores usually appear at the point of infection, but in some cases no connection with an injury could be seen. Although the non-occurrence of any obvious wound or dead stub cannot be taken to imply the absence of infection through a wound, it is possible that the fungus gained its entry into the tree through some minor injury, which exposed the living tissues for a time long enough for them to be attacked. It will be shown later that *P. hispidus* is capable of attacking young sapwood, though it is normally a heart-rotting fungus.

Trees attacked by this fungus show little sign of its presence; at any rate trees bearing sporophores and showing little evidence of weakening are common. Later on, however, they begin to die back, and eventually death ensues.

One of the most important points of interest about the fungus is its possible connection with that condition of ash known as "brashness[1]." Apparently sound ash will often be found to break under a strain much below the normal, the fracture being of a peculiar brittle nature. In many cases this "brashness" is obviously due to the poor quality of the timber used, since ash which is slow grown and which contains an unduly large proportion of the weak spring wood will naturally be weak. In some instances, however, the "brash" fracture occurs in wood which is anatomically sound; that is to say, wood that is indistinguishable from that of high strength by the microscopic examination. A number of such examples have been examined, and in every instance the presence of a fungus has been demonstrated in the wood, the hyphae being very sparsely scattered, though they are evenly distributed. As yet no cultures have been obtained from such samples, the inference being that the fungus has died out in the seasoned timber. As sporophores have

[1] The term "brashness" arose in America, and is now in common use in the timber trade of both this country and the U.S.A. In this paper its use will be restricted to that condition of timber, however caused, which produces a peculiar "carroty," short, fracture.

never been seen by the writer on fallen branches of ash or on dead trees, it is possible that the conditions for growth of this fungus are such that it dies out subsequent to felling. As far as I am aware, no record exists of the fungus being found on dead wood.

Baxter states that mycelial wefts are found in trees decayed by this fungus up to a distance of 6 ft. from the last stage of visible decay or discoloration, and that the wood in this region is infected with mycelium. The fact that mycelium can be demonstrated in wood at a considerable distance from the visible decay has been well established by Hubert (10), and observations made on decayed ash trees confirm his results. Hence a proportion of the timber from any infected tree, while *apparently* sound, may yet contain a considerable amount of fungal mycelium.

Unfortunately, in the present state of our knowledge we do not know the precise effect of a fungus on wood during the early stages of decay, but from general observation it seems probable that a considerable proportion of the wood from a tree which is slightly decayed should be suspect. While in some circumstances the decrease in strength may not be of any great practical importance, yet at other times, for example in aeroplane construction and in the manufacture of tennis rackets, strength is the very factor for which the wood used is selected. It is well known that in the trade, ash, particularly English-grown ash, is highly valued for its great toughness and elasticity.

The fungus most commonly found on ash is undoubtedly *P. hispidus*. *Fomes fraxineus* may sometimes be met with, but it is not nearly so frequent in occurrence, being listed as "uncommon" by Rea. If, therefore, any definite relation between "brashness" and fungal infection should be found to exist, the fungus chiefly concerned will probably prove to be *P. hispidus* with *F. fraxineus* occupying a subordinate position.

This paper is the result of a cultural study of this fungus, and of its enzymes, preliminary to a more general investigation of the problem of "brashness" in timber.

Cultural characteristics.

Polyporus hispidus grows readily, though slowly, on all the ordinary culture media, solid or liquid, though less rapidly on synthetic media such as Richard's. For this work ordinary 2 per cent. malt agar was found to be most satisfactory, and was used throughout. The fungus at 25° C. covers an ordinary 4-inch petri dish of this medium in about one month.

On agar the mycelium forms a thick mat on the surface of the medium, the stout hyphae forming a plush-like growth overlying a dark brown

skin-like membrane formed in contact with the agar. The mycelium is bright antimony yellow—lighter in the younger portions and shading into buckthorn brown in the older parts; all the colours used in the description of the fungus are according to Ridgeway. No traces of fructification have been observed on solid media although the usual expedients of varying the temperature, altering the humidity, changing the concentration of food materials, etc., have been tried. Baxter obtained similar negative results on attempting to induce fruiting.

In liquid media the fungus makes very good growth, especially on turnip extract. This extract was prepared by autoclaving cubes of turnip without the addition of water and expressing the juice, which, when filtered and sterilised, proved to be an ideal medium for the fungus, especially when large quantities of mycelium were required.

On this the young mycelium is mostly sub-aerial, forming a dense woolly mat of upright stout hyphae floating on the surface of the liquid. These are colourless or else a very pale yellow. In fact, on any medium where the growth of the fungus is sufficiently rapid, the coloration is never produced until staling has set in and the growth has been somewhat retarded. This also appears to be true for the majority of wood-destroying fungi, some of the more rapidly growing of which do not assume the characteristic coloration until after a considerable lapse of time. It almost appears as though some substance produced in staling is responsible for the coloration of the fungus.

On turnip extract the coloration begins to develop after about a week, starting in the centre and spreading rapidly all over the colony until the whole is a very beautiful pale barium yellow. After this stage growth slows down, the colony becomes very much more compact, and a considerable development of submerged mycelium takes place. At the same time the colour becomes much more vivid, and deeper in tone (antimony yellow), while patches of buckthorn brown appear on conical or hemispherical protuberances arising from the level mycelial mat. These often become hispid, and sometimes approach Dresden brown in colour. They were noted by Baxter, who mentions their frequent occurrence and their hispid character. In no case do they give rise to fruit bodies, as the single fructification which was produced in culture arose very rapidly from a younger perfectly level mycelial mat.

Eventually the fungal colony becomes a widespreading and gelatinous mass of submerged mycelium, bearing on its upper surface a dense felted mat of aerial hyphae, which frequently exudes dark sticky drops of a very astringent taste. The colouring matter is almost confined to the aerial

hyphae which are very uniform in size. They are of a bright yellow colour under the microscope, the colour being mostly in the cell wall.

On examining the younger stages of the submerged mycelium it was found that there was a pronounced tendency for the hyphae to branch very freely, generally dichotomously, and for these branches to become highly vacuolate. They then swell very considerably at the vacuoles, especially at the tip of each branch; in this way little clumps of denser appearance begin to occur in the mycelial mass. These clumps appear

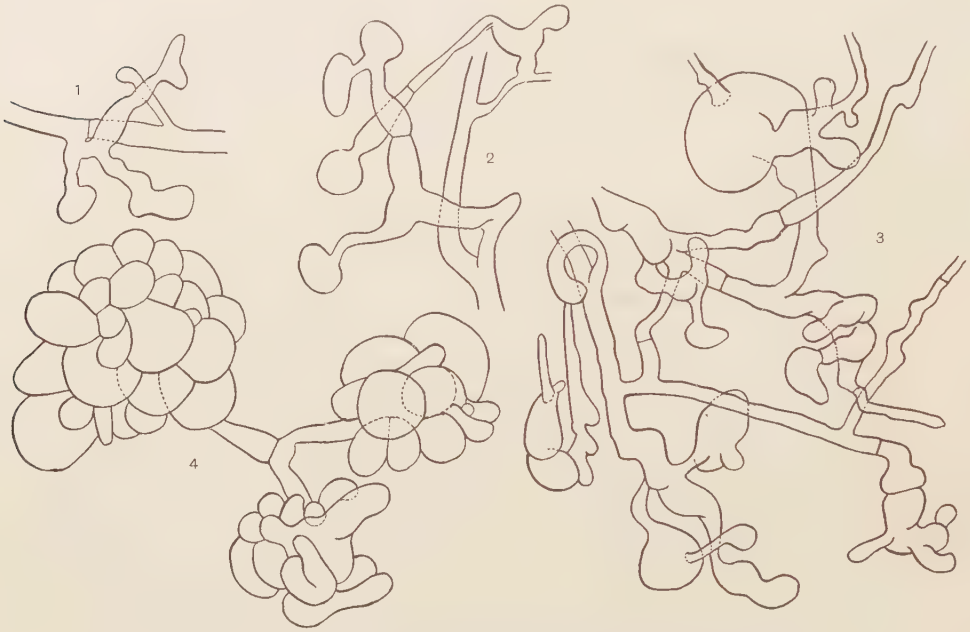

Fig. 1. Formation of plectenchyma by mycelium of *Polyporus hispidus* in liquid medium.

with some regularity, scattered throughout the felt of hyphae, and, when the mass is examined under a low power, look like clumps of large yeast cells. They spread and get denser, eventually joining up and forming a mass of vesicles which, pressing closer together as the swelling increases, form a kind of plectenchyma. Soon the appearance of hyphae is entirely lost, the only thing to be seen being a mass of small bladders resembling bunches of grapes in surface view.

It is noteworthy that no clamp connections have been found in culture, either on solid or on liquid media.

Production of fruit bodies in culture.

As has already been stated, the production of fruit bodies of *P. hispidus* has, in general, been absent, no expedient succeeding in inducing fruiting. However, one liquid culture suddenly produced a large irregularly hemispherical protuberance, studded with dark brown drops, which looked as though they were being excreted from a pore surface. The protuberance, too, was distinctly hispid in patches, and its general appearance was not in the least like the smaller ones referred to previously. It also differed in the rate of growth, since on Saturday midday the colony was perfectly normal, and on Monday morning the protuberance had reached the dimensions given below. After being photographed the mass was removed from the Erlenmeyer flask in which it was grown. It had become so tough that it was necessary to break the glass in order to remove the fructification. The fruit body was about 4 in. by 3 in. in extent, and 2 in. thick, the pore surface being about 3 in. by $2\frac{1}{2}$ in.

On examination it was found that a definitely organised pore surface was present, with large pores averaging 0·7 mm. in diameter and about 6 mm. deep. However, there was no hymenium lining the tubes, or at any rate no well-organised one, while no spores could be found. Apparently the fruit body has just entered on its final stage of development, and was removed from the flask too soon.

The culture in question was one of many put up to obtain a large quantity of mycelium for an investigation of the enzymes, and had been kept for most of the time in an incubator at 25° C., though it had occasionally been removed and kept at room temperature for some days owing to lack of incubator room.

Westerdijk (19) states that fluctuations of temperature often induce fruiting in the higher Basidiomycetes. This explanation does not seem available here, since all the flasks in this particular batch had been subjected to the same temperature variations: also, deliberate alterations in temperature have produced no sign of fruiting.

Growth on inoculated wood blocks.

In order to trace the development of the hyphae in the wood of ash, small blocks were placed in a flask, autoclaved with the addition of water, inoculated with *P. hispidus* and incubated at 25° C. Some external growth was made, enough to show that all the blocks were being attacked, but not enough to be very obvious; most of the mycelial development was in the substance of the blocks.

At the end of four months samples were removed and sectioned on a Reichert microtome. As they were fully imbibed with water they sectioned readily enough without demineralisation, when a very sharp knife (Jung) was used. Sections 9μ to 10μ were easily obtained, and these were stained with safranin and picro-aniline-blue. This method of staining fungal hyphae in wood is due to Mr K. St G. Cartwright, of the Forest Products Research Laboratory and will shortly be described by him. The protoplasm of the fungus, and to a less extent the fungal cell wall, took up the blue stain, forming a brilliant contrast with the red lignified walls of the wood.

The blocks were 1·5 cm. × 2 cm. × 3 cm. and in all those sectioned hyphae were present in the centre of the blocks. As this gave a rate of penetration much greater than that recorded by Baxter, another experiment was set up to gain more accurate information.

Growth of hyphae.

The greatest development of the hyphae at this stage of attack was in the medullary rays, nearly all the cells of which contained hyphae, branching freely but never forming a thick weft in the cells such as is formed by some species of fungi, *e.g. Fomes ribis*. The hyphae in the rays were very granular and dense, being full of protoplasm and obviously well nourished. They varied greatly in diameter, some being quite thick (3μ to 4·5μ), while the majority were about 2μ. Baxter gives 7μ as the largest hypha that he has observed. Hardly less numerous were the hyphae in the xylem parenchyma. Here the diameter was much more uniform, being on the average 1·5μ. The majority of the hyphae were of this size, though some were very fine and hyaline. No clamp connections could be observed.

At this stage of decay there was very little development of the fungus in the fibres, nor, apparently, in the vessels, as no hyphae were seen in the latter even on close examination of many sections. Later it will be shown that the absence of mycelium in the vessels is only apparent, and that in reality the vessels are attacked very early in the history of decay. The process of sectioning, however, tears the hyphae from the lumina of the vessels, suggesting that the hyphae avoids the vessels. Apart from this it appears that the fungus makes its greatest development in the medullary rays and in the xylem parenchyma, owing possibly to the more easily available food supply. In the blocks the typical decay was not produced, though a slight discoloration was seen at the periphery. No marked alteration of the staining reactions of the cell-wall was

produced, and there was apparently little effect on the wood. The general impression from examination of the sections and of the wood in bulk was that the fungus was living at the expense of the organic and other debris in the cells, and had only just begun to break down the cell-wall.

After decay had progressed for six months the distribution of the hyphae had altered very considerably. The vessels were choked with a thick weft of mycelium, though this was generally lost in the process of sectioning. The medullary rays and the xylem parenchyma were full of hyphae, much resembling those in the wood decayed for four months, but rather more abundant. The fibres by this time were vigorously attacked, any section showing many hyphae ramifying in the cells. These hyphae are quite large in diameter, full of protoplasm, and apparently active and well nourished. They bored freely in the walls, without reference to the position of any pits. It is peculiar that in the early stages of decay penetration should be almost entirely by means of the pits, while later on it should be by "bore-holes," obviously the result of enzyme activity, while pits, in general, are ignored as a means of passage from one cell to another.

An interesting feature of the bore-holes is that they are frequently formed in rows down one cell-wall by a single hypha, which winds through the wall and back again in very much the same way that a thread does in cloth. In fairly advanced decay the damage done to the cell-wall merely by bore-holes appears to be considerable, without taking into account any general weakening of the tissues which is presumably produced by the enzymes of the fungus acting more or less evenly all over the walls.

In no case were any clamp connections, medallion hyphae, or "buckles" observed in wood decayed by *P. hispidus*.

P. hispidus does not usually form "zone-lines" in wood, though Baxter records that in some instances brown lines are formed during decay. In the blocks decayed for six months dark lines were formed in the more decayed portions. These, under the microscope, can be seen to be due to a dark brown material filling the cavities of the cells. In suitably thin or bleached sections this material can be seen to consist of solid masses of old hyphae, large in diameter (7μ to 8μ), and thick-walled; they have lost their protoplasmic contents, and their walls have turned a deep brown. These dead hyphae are not confined to the cells in the "zone-line," but can be found mingled with the normal hyphae in the cells near that line. With advancing age, the normal hyphae appear to

pass over into the thick-walled type, or perhaps when there is no further nutriment in the wood.

An attempt was made to get a more accurate idea of the rate of penetration of hyphae through wood. For this purpose blocks, 5 cm. × 1 cm. × 1 cm., were cut from sound wood in such a way that the long axes were

1. Longitudinal;
2. Radial in the tree.

One dozen of each kind were put up. They were coated with sealing-wax all over, with the exception of the smaller ends, and then re-coated with colloidin. As sterilisation by autoclaving was impracticable, because of the certainty of the wood being altered by the treatment, and also because of the effect on the coating, the blocks were sterilised at 60° C. for 12 hours in a saturated atmosphere. They were then kept for one month in a saturated atmosphere in order to become uniformly imbibed, and then inoculated at the small ends with mycelium of *P. hispidus*.

After six months the blocks were removed and the sealing-wax removed. The decay had spread down the blocks with some rapidity, the wood nearest the point of inoculation being bleached to a pale yellow colour, and being obviously softer and more spongy than the unaltered wood at the distal end of the block, or the slightly attacked wood in the intermediate portions. This lighter portion of the wood extended 7 mm. on the average from the point of inoculation in those blocks in which the path of the fungus was across the growth rings, and to a slightly greater distance in the others, though in these it was not at all sharply delimited. Small pockets of the yellowish rot could be traced to a distance of 2 cm. from the point of inoculation.

The surfaces of the blocks were trimmed off very carefully in a microtome, great care being taken to ensure the surface being smooth and plane, and the blocks mounted whole on a slide. They were then examined under a 16 mm. objective and a Beck ring-illuminator. By this means hyphae could be seen in the vessels as an open, brilliantly illuminated network, standing out with great vividness against the black background of the vessels. Mycelium could also be seen in the fibres and sometimes in the rays. The visibility of mycelium in the rays is of rare occurrence, not because of any lack of hyphae, but because there is generally a cell-wall between the mycelium and the surface of the section.

By this means an estimate of the distance to which the fungus had penetrated could be made, especially as the zone containing the fungus

in the vessels was very sharply delimited from the unattacked wood, on both the transverse and the longitudinal faces of the blocks.

The distance traversed by the fungus in a radial direction, *i.e.* across the growth rings, was 3·2 cm., no block differing more than 1 mm. from the average. As wood is such a heterogeneous substance the unexpected uniformity of these results is of interest, although every precaution was taken to equalise the conditions in the various blocks. Baxter obtained a longitudinal rate of penetration of 1 mm./month, but in his case the material was young ash sprouts, so that the two results are not comparable. Unfortunately the fungus had penetrated completely through the blocks in a longitudinal direction, so that no definite data could be obtained. The results are, therefore:

Penetration across growth rings	...	5 mm./month.
Penetration parallel to the grain	...	7 mm./month, at least.

In order to prepare wood for observation by means of the ring illuminator it is to be emphasised that the surface must be rendered perfectly plane and smooth by a heavy razor, mere smoothing by a knife, plane, or scalpel not being sufficient. The open ends of the vessels and fibres must be cut cleanly across without distortion, and debris must not be allowed to fall into them. These conditions are admittedly difficult to realise, especially with some woods, but the results repay the labour expended, as it is possible to get a very accurate idea of what the fungus is like in the wood, in a way that is impossible by the examination of sections, which of course should be used as well. I have found that the easiest way of preparing the blocks is to clamp them securely in a sliding microtome, and then to take shavings from the face to be examined, using a very sharp, very heavy razor, set at an extreme angle to give a long drawing stroke. In this way a face can be prepared which is suitable for observation by reflected light.

Unfortunately it is almost impossible to obtain a successful photomicrograph of this, as the hyphae are generally some little way down in the vessels, and if they are in focus the wood is not, and *vice versa*. Also, owing to the lack of focal depth of an objective, it is generally impossible to focus the whole of the surface of the wood. When working, however, this is of no moment, as one automatically moves the fine adjustment and so obtains a composite image of the surface of the block.

In the decay of ash by *P. hispidus* the mycelium ramifies to a considerable extent in the vessels, which appear to be the paths of the fungal advance in the longitudinal direction, as the medullary rays are in the

radial. The vessels are large and when sections are cut it is rare for any hyphae to remain in them. They are either torn out by the microtome knife, or are washed away during the staining process, unless some method of embedding is used. The results are then generally unsatisfactory from other points of view, to say nothing of the inconvenience and the length of time involved. It is, therefore, easy to reach the conclusion that the hyphae are avoiding the vessels for other parts of the wood, whereas the exact reverse is the case. Under the ring illuminator the hyphae can readily be seen in the vessels, and a good general idea of the rot obtained. The magnification obtainable is not great, but the definition is very good, and the full resolving power of the lens is utilised. Owing to the great advantage of viewing a block of wood with the mycelium *in situ*, it is urged that some form of ring illumination should always be used in the description and investigation of decay in wood, at any rate in woods which have large pores.

The parasitism of *Polyporus hispidus*.

It is naturally of considerable importance to determine whether *P. hispidus* is an obligate saprophyte, or whether it can attack cells which are still living. If the latter is the case the tree can be attacked through minor injuries, while if the former the attack must be through branch stubs or similar defects which expose the dead heart-wood of the tree.

Baxter succeeded in infecting freshly cut black ash sprouts, containing only sap wood, with the mycelium of the fungus, finding that it attacked the medullary ray cells readily, as well as the wood elements. He also succeeded in carrying out successful inoculations of young trees, and in tracing the mycelium some little distance into the wood.

Young ash trees, 10 years old, at Oxford, were inoculated in June 1927 with the mycelium of *P. hispidus* as follows: a T-shaped slit was cut in the bark with a sharp scalpel, and the flaps of bark pulled back. Actively growing mycelium was then placed on the wound, and the flaps pressed firmly in position. The wound was then covered with viscous paper bound tightly in position with wool, or with grafting wax. After three weeks some of the trees were removed and sectioned. The mycelium could be seen pressed closely in contact with the wood, and longitudinal sections showed the presence of young hyphae sparingly scattered in the zone immediately behind the point of inoculation. Most of the hyphae were in the medullary rays, a few being in the parenchyma of the wood. These were small ($1{\cdot}5\mu$), densely granular, and apparently actively growing.

Three months after inoculation other trees were removed and sectioned in a similar manner. The wound had completely healed by this time, and the original inoculum could still be seen included in the young wood. Hyphae were readily found in the medullary rays, xylem parenchyma, and vessels, immediately behind the point of inoculation. They were larger, on the whole, than those seen in July, and were obviously well nourished. They had not spread far through the wood, the greatest distance noted being 2 mm., but were quite numerous. The general impression given was that the fungus, having gained a foothold in the young wood, was flourishing there in a small area, and was invading fresh tissues slowly.

From these results, and from those of Baxter, it appears certain that though *P. hispidus* is normally a saprophyte, in that it attacks the heartwood, yet this saprophytism is facultative, since it can, and no doubt does, attack living cells.

The penetration of the cell-wall.

In the blocks subjected to the action of *P. hispidus* for four months, penetration was almost entirely by means of the pits, with which the cells of the medullary rays and the xylem parenchyma abound. Penetration of the cell-wall in a ray cell has not been observed, and only occasionally in a cell of the xylem parenchyma. This is true, not only of the blocks decayed for four months, but also of those rotted for longer periods. In other words, penetration has never been observed other than by pits in either

1. A medullary ray cell.

2. A xylem parenchyma cell (except in the wall bordering on the fibres).

3. A vessel.

This is interesting with regard to a statement by Hubert, to the effect that wood-destroying fungi generally exhibit a complete indifference to the pits, often passing through the wall very near them. While this is, no doubt, true for some fungi, it is not always so, since in this case the fungus appears to prefer the pits as a passage way from one cell to another, and yet it is capable of causing a very considerable decay of the wood.

In all, 27 sections of the four months' rotted wood were examined, and in these penetration other than by pits was only noted three times. In none of these could the method of penetration be seen clearly, though

it appeared to conform to that seen in blocks at a more advanced stage of decay.

In the blocks rotted for six months, under what were probably better conditions for the growth of the fungus, penetration could be seen very frequently, and all stages could readily be observed. In general it is of

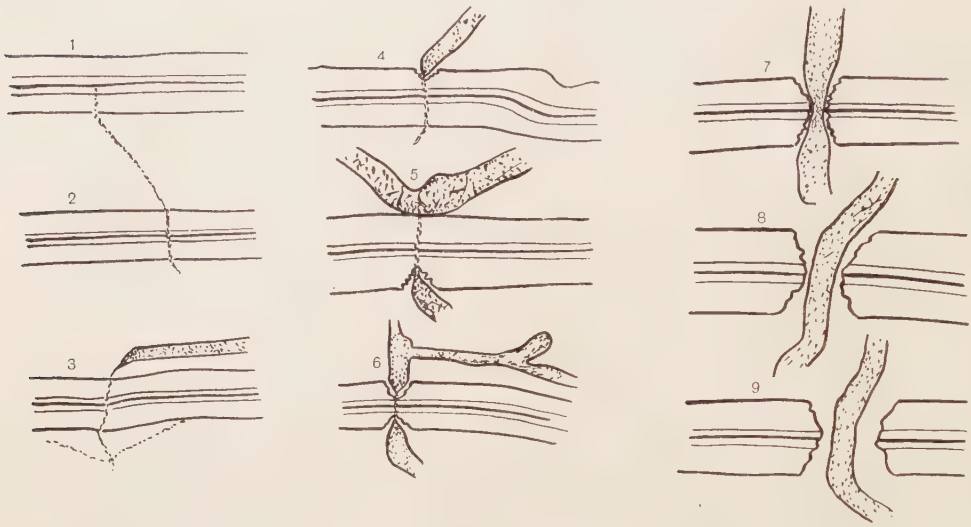

Fig. 2. Penetration of the cell-walls of ash by hyphae of *Polyporus hispidus*.

1. Fine hypha half through cell-wall.
2. Fine hypha completely through cell-wall.
3. Hypha thickening up at one end of the bore-hole.
4. Erosion beginning at one end of the bore-hole.
5. Erosion in progress at one end of the bore-hole. Hypha thickened on both sides of the cell-wall.
6. Erosion in progress at both ends of the bore-hole.
7. Bore-hole much enlarged. Hypha passing through with but slight constriction.
8. Bore-hole still further enlarged. Unconstricted hypha passing through bore-hole very slightly larger than itself.
9. Final stage. Unconstricted hypha passing through bore-hole much larger than itself.

two distinct types, one being common, while the other is much more rare. These are:

1. Penetration by means of the tip of a young hypha, which subsequently thickens and erodes the cell-wall.

2. Penetration by means of a peg sent out from an older and thicker hypha.

Penetration by the tip of a young hypha.

This appears to be the normal mode of penetration, and all stages may readily be seen in suitably stained sections. For this work the safranin-aniline-blue combination was found to be very useful, due to the brilliant contrast obtainable between the hypha and the wall which it is penetrating.

The first stage seen is when a young hypha, so fine that it is merely a line of small protoplasmic granules under a $\frac{1}{12}$th objective, comes in contact with a wall. It bores straight through, taking the usual shortest path, *i.e.* perpendicular to the cell-wall, with no change of diameter at all. The penetration here must be enzymatic, since it is hardly conceivable that so fine a thread, with no appressorium, could penetrate a thick lignified cell-wall by mechanical means. It is unusual and interesting that in several cases observed (figured in No. 3 and to a less extent in No. 4) the hypha turns aside from the normal path. It was at first thought that this was due to some distortion of the cell-wall caused by sectioning, but more detailed observations showed that this was not the case, since other hyphae penetrating the same cell-wall nearby were not deformed in a similar manner.

After penetration, the young hypha thickens, until it has reached approximately its mature size. The portion in the wall undergoes no change, remaining too small to be measured. At this stage the hypha is about $1{\cdot}5\,\mu$ in diameter, the two parts being joined by the very fine thread in the cell-wall. After this an erosion takes place round *one* end of the thin thread in the wall, followed soon after by a similar erosion at the opposite end. As this increases, two cone-shaped openings are formed in the wall with their axes along the thin fungal thread. As the cones increase in size the hypha swells up, the swelling of the hypha and the enlargement of the cones going on simultaneously, until the apices of the cones meet and pass. This produces a large bore-hole, with an unconstricted hypha passing through. By this time the outer opening of the bore-hole is much larger than the hypha. Erosion proceeds still further, however, the final stage being reached when the hypha lies in a hole the minimum diameter of which is three times that of itself, the maximum being five times.

Penetration by means of a peg-like outgrowth.

This is of quite rare occurrence, but may sometimes be noted in wood which has been considerably decayed. It differs from the previous type in that it takes place when an older and thickened hypha comes to lie

against a cell-wall. From the hypha a peg is put out, of approximately the same diameter as the tip of the normal type, which penetrates as before. Presumably the thickening up of this peg goes on in the same manner as that described for the previous type, but it has not been observed.

A good, though brief, review of the literature of cell-wall penetration, and an historical summary, is given by Hubert, dealing more especially with *Trametes pini* as a type of fungus whose enzymes are secreted solely by the tips of the hyphae, the bore-holes consequently never enlarging. Some of the fungi which exhibit penetration resembling that of *P. hispidus* are:

Fungus	Observer
Polyporus Fulvus	Hartig
P. nigricans	Lindroth
P. betulinus	Lindroth
P. laevigatus	Lindroth
Lentinus lepideus	Buller
Fomes igniarius	Hubert
F. ribis	Nutman
Trametes robinophila	Hubert
Stereum hirsutum	Nutman

The enzymes of *Polyporus hispidus*.

In spite of the great importance of the higher fungi in the decay of wood, surprisingly little work has been done on their enzymes. Seeing that these enzymes are the armoury of the fungus, it behoves us to know as much as possible about them. Zeller (20) gives a brief summary of the work done on these enzymes in his paper on those of *Lenzites saepiaria*.

All work on fungal enzymes, if it is to be at all controlled, necessitates the preparation of an enzyme suspension from the fungus concerned. The means of obtaining such suspensions are often by no means free from objections. Some workers, such as Bourquelot (3), Herissey (4), and Buller (7), have expressed the juice from the sporophores of the fungus, since the sporophore affords a ready means of obtaining a large quantity of pure mycelium of the fungus under investigation. This juice has been used either directly, as an enzyme suspension, or has been treated with alcohol to precipitate the enzymes which have been re-suspended in water.

This can be objected to on two grounds: Firstly, because the mycelium in question is generally old, and, as Brown (6) has shown, the enzymes are naturally concentrated in the tips of the young actively growing hyphae. In the case where the sporophore is young and actively growing this

objection is not valid, though the second one would apply. The second objection is that the sporophore is a fruit body, and the enzymes present are not necessarily an indication of the full complement in the young vegetative mycelium. This has been well demonstrated by Zeller, in an investigation of the enzymes of the mycelium and sporophore of *Lenzites*, where he found that many enzymes, notably invertase, diastase, tannase, and cellulase are abundant in the mycelium and are scanty or absent in the sporophore. In some instances, such as the oxidases, the converse may be true.

The point is that the mycelium of the fungus may be divided into two physiologically distinct units, the first being purely vegetative and metabolic, equipped with the enzymes necessary for its particular type of metabolism, while the second is the mycelium building up the fruit body, which is not necessarily equipped for the same physiological processes as is the vegetative part of the plant. Hence, in order to get any idea of how the fungus employs enzymes in its particular mode of life, it is necessary to use the vegetative mycelium as a source of enzymes, since many such enzymes might be absent in the sporophore and would be missed if a sporophoral extract was used.

Zeller used an extract from sawdust on which the mycelium had been cultivated for a period of seven months. In such a case much of the mycelium would be old and relatively poor in enzymes. He also used an extract from the dried sporophores.

In this work on *P. hispidus* endeavour was made to grow the fungus as rapidly as possible, so that the extract could be made from young mycelium containing a large proportion of enzymes. Since the plant does not form spores in artificial culture, the method evolved by Brown for *Botrytis cinerea* could not be used and a modification of it was adopted. The mycelium was grown in large Erlenmeyer flasks in turnip extract, which was found to be most favourable for rapid growth. The flasks were inoculated with six or seven pieces of mycelium, in order to provide several centres for growth. All the cultures were incubated at 25° C. In about one month a large quantity of mycelium was produced. This was removed, washed for some time in running water, dried as thoroughly as possible between sheets of blotting paper, and the process completed *in vacuo* over recently fused calcium chloride. The dried mycelium was then ground to powder in a mortar, and kept for future use. To make the enzyme extract 10 gm. of this powder, containing about 5 gm. of mycelium and about 5 gm. of sand, was extracted with 100 c.c. of distilled water for 24 hours. The aqueous extract was filtered, and toluene was

added as an antiseptic. This will be referred to as enzyme extract No. 1.

In some cases the enzymes were precipitated from this by the action of three volumes of 95 per cent. alcohol. The flocculent precipitate was caught on a filter-paper, washed with alcohol, and the suspension reformed by allowing the paper to stand in distilled water for a few hours, with occasional gentle agitation. This enzyme suspension was often used at the same time as No. 1, and in every instance gave identical results.

Another extract was prepared from the fresh mycelium. After washing it was immediately transferred to a mortar, where it was ground up with sand as rapidly as possible. The pasty mass was then extracted with water and filtered, toluene being added to the filtrate as an antiseptic. It will be referred to as enzyme extract No. 2. In all instances where Nos. 1 and 2 were used at the same time the action was identical qualitatively, and more or less the same quantitatively, except with the catalases, where much greater effervescence was given with No. 2.

These enzyme suspensions, judging by results, are much more active than those used by other workers on the enzymes of the Basidiomycetes. For example, the guaiacum reaction for oxidase, which took two hours to develop with Zeller's extract, took place in 4 minutes with extract No. 1, and in $3\frac{1}{2}$ minutes with extract No. 2. This might appear to be due to some excess of oxidase in *P. hispidus*, and perhaps this does contribute somewhat to the short times recorded in this particular test. Generally, however, the time taken to produce a given reaction was shorter than that recorded elsewhere, especially in the tests for emulsin, hemicellulase, and diastase.

Only the principal enzymes were investigated.

Emulsin. This enzyme has been known since 1837, and was found in 1894 in 34 species of fungi by Bourquelot, so that it appears to be generally distributed. Its importance in decay is that in the decomposition of wood, whether coniferous or otherwise, it is probable that various glucosides are set free.

Zeller found that in the decay of pine wood by *Lenzites*, vanillin was formed in fairly large quantities if the blocks were kept saturated with water. It is therefore probable that these glucosides, such as populin, arbutin, amygdalin, and coniferin, are broken down by the fungus and are partly converted to glucose, which is easily assimilated.

A 1 per cent. solution of amygdalin was used as a substrate, and 10 c.c. portions were placed in test-tubes. To three of these were added 1 c.c.

of enzyme extract No. 1 and to three 1 c.c. of No. 2. One of each of these was boiled. To three other tubes were added 1 c.c. of distilled water. To all were added toluene as an antiseptic. The tubes were incubated at 25° C. After 18 hours all the regulars gave a strong odour of benzaldehyde. The contents of the tubes were filtered off and tested for reducing sugars by means of Fehling's solution. All the regulars gave a positive result, while the controls gave a negative result. This shows that the mycelium of *P. hispidus* contains the enzyme emulsin.

Diastase. This enzyme has previously been reached in *Fomes annosus* by Hartig, and in other higher fungi by Bourquelot, especially in *Polyporus sulphureus*. It occurs in *Merulius lacrymans*, *Polyporus squamosus*, and *Armillaria mellea*, and Zeller has found it in both the mycelium and sporophores of *Lenzites saepiaria*.

A solution of soluble starch was used as a substrate in testing for this enzyme. It was prepared as follows:

2 gm. of starch in 100 c.c. of distilled water was brought to the boil, with constant shaking, and poured into 300 c.c. of hot distilled water. The resulting solution was then boiled for 2 hours under a reflux condenser, cooled, and brought up to 500 c.c.; to this was added toluene as an antiseptic.

To portions of the above solution in test-tubes were added: (1) enzyme suspension No. 1; (2) enzyme suspension No. 2; (3) enzyme suspension No 1 (boiled); (4) distilled water. They were incubated overnight at 25° C. and tested with Fehling's solution in the morning. Nos. 1 and 2 gave a considerable amount of reduction, while the two controls gave clear tests. Hence the mycelium of *Polyporus hispidus* contains the enzyme diastase.

Invertase. A 1 per cent. solution of cane sugar was used as a substrate, and to portions in test-tubes were added the enzyme extract, the extract boiled, and distilled water. The tubes were incubated at 25° C. as usual. No action was observed until after 18 hours, and even then it was very slight.

The mycelium undoubtedly does contain the enzyme *invertase*, though in very small quantity. This is more or less to be expected, since it is difficult to see where a wood-destroying fungus could obtain sucrose. The only possible place appears to be in the sap wood, where sucroses are probably present. Both Baxter and the writer have shown that *P. hispidus* can attack sap wood.

Cytohydrolysing enzymes. Under this general term will be included those enzymes which break up the higher carbohydrates, such as those which compose the cell-walls of plants. They include ligninase, cellulase, hemicellulase, pectinase. It has been known for a long time that fungi can effect a change in the properties of the lignified cell-wall, the first to call attention to this being Hartig, who showed that many species of fungi can break down the cell-walls of wood, and who stated that this was brought about by the action of a fluid secreted by the fungus.

Basidiomycetes are the fungi most concerned with the breaking down of the cell-walls, though under suitable conditions other groups can also attack wood. Miyoshi(12) and Marshall Ward(18) both showed that *Penicillium* spp. could attack wood, and the writer has frequently isolated species of *Penicillium* from woods which have been quite considerably decayed, and which, apparently, contained no other fungus. This has been especially noted in connection with a *Penicillium* which has been isolated, several times, from wood attacked by *Xestobium rufo-villosum.*

While it is relatively easy to form the conclusion that a fungus which breaks down lignified walls must possess a lignin-splitting enzyme, it is by no means easy to devise a test by which the ligninase can be demonstrated.

Czapek(9) found that when decayed wood was extracted with hot alcohol or benzene, a resinous substance could be isolated from the alcohol, which he called "hadromal," probably an aldehyde, possibly coniferyl aldehyde. This he obtained from wood decayed by *Merulius lacrymans*, *Polyporus adustus*, *Pleurotus pulmonarius*, *P. ornatus*, and *Armillaria mellea.* From sound wood he obtained relatively little hadromal. He also noted that normal lignified walls show no sign of the cellulose reaction, but that when they are slightly decayed the zinc-chloriodide test for cellulose gives a positive reaction, though the walls show no signs of breaking down. He also found that the hadromal solution would give a pink coloration with phloroglucin and hydrochloric acid.

His conclusions were that the cell-wall is composed of a "cellulose-hadromal-ether." By the action of the enzyme produced by the fungus this ether is broken down, setting free the hadromal, which is soluble in hot absolute alcohol, and also liberating the cellulose, which can then give its characteristic colour reaction.

He prepared an enzyme suspension from *Merulius lacrymans* and *Pleurotus pulmonarius*, and incubated shavings in it for a fortnight. The extract from these shavings gave a strong hadromal test, and the cell-

walls of the shavings gave a purple reaction with zinc-chlor-iodide. The extract lost its activity when boiled.

Apart from the theoretical question of the validity of Czapek's conception of the lignin complex, it appears that a convenient test for an enzyme attacking lignified tissue is the reaction of the alcoholic extract of the decayed wood with phloroglucin and hydrochloric acid. Zeller used Czapek's test for "hadromase" as follows: he placed 1 gm. of shavings of *Pinus echinata*, previously soaked in distilled water, in test-tubes, and to these he added the enzyme dispersions. After 15 days the dispersions were poured off, and the shavings boiled in absolute alcohol, and the extract tested with phloroglucin and hydrochloric acid. The shavings acted on by the enzyme gave pink extracts, while the controls gave clear, colourless tests. Hence Zeller concluded that "these reactions show conclusively that hadromal is split off in the presence of an enzyme suspension from the mycelium of *Lenzites saepiaria*."

He attacks Czapek's nomenclature of the enzyme (hadromase) on the ground that it attacks lignin, not hadromal, and proposes that the term ligninase should be adopted. As this agrees with the terminology of Duclaux, which is in general use, it will be used here.

Baxter attempted to apply Czapek's test to distinguish between sound and apparently sound wood of *Fraxinus nigra* attacked by *P. hispidus*. He found that the reaction was given more or less equally by both the sound and the decayed wood. He prepared enzyme extracts in the usual way, and tested the sound wood against that acted on by the enzymes, but obtained no significant difference. His conclusion was that the results of Czapek's test cannot be relied upon, at any rate in the case of the hard woods.

The great discrepancy between the accounts of the reaction by these two workers makes it necessary that the matter should be cleared up. The fact that they worked with different woods has to be taken into account. In order to do this samples of sound wood of the following species were collected, and shavings made from them: *Pinus sylvestris*, *P. excelsa*, *Abies pectinata*, *Fraxinus excelsior*, *Castanea sativa*, *Fagus sylvatica*, *Salix* sp.

Small samples of these shavings were boiled for 10 minutes in absolute alcohol, and the extract carefully filtered, in order to remove small particles of wood. The alcoholic extracts were treated with phloroglucin and hydrochloric acid, and in every case a fairly deep red colour was produced. Wood of *Fraxinus excelsior* which had been decayed by *P. hispidus* for 5 months was treated in a similar manner. The difference

in the tests for sound and decayed wood was so small as to render the test valueless.

Hence it is clear that sound wood of the preceding species contains enough alcohol-soluble material, giving the phloroglucin test, to invalidate any estimate of the power of a fungus to attack the lignified tissues of the wood.

Attempts were made to treat the wood in such a way as to extract this substance. It was found that this can be done by boiling the wood in several changes of distilled water. When dried, and subsequently extracted with boiling alcohol in the usual way, the alcoholic extract is free from such substances giving the phloroglucinol reaction. Owing, however, to the probability that the hydrolysis of some of the wood compounds might take place during the boiling, this method was abandoned and the following adopted.

Shavings of the various woods were placed in large boiling tubes, and extracted with absolute alcohol under a reflux condenser. The alcohol was renewed from time to time, till the last traces of the substance giving the phloroglucin test was removed. That is, until the last extract did not given any perceptible coloration with phloroglucin and hydrochloric acid. Some of the woods gave a colourless test after three periods of extraction of 1 hour each, while the others needed as many as seven extractions of a similar time to remove all the "hadromal." The shavings were then dried, and kept in an air-dry condition as samples of wood with the hadromal of Czapek removed. Some of these shavings were soaked in distilled water for some time in order to remove the water-soluble materials, dried, and placed in boiling tubes. These were plugged, and sterilised by being subjected to a dry heat of 105° C. for 2 days. This temperature was chosen because no change in the chemical nature of wood takes place until the temperature exceeds this figure. Sterile water was then added to the tubes, and they were put on one side for several days until the wood had absorbed all the water it could.

The moist shavings were then transferred to the surface of agar slants which were covered with actively growing mycelium of *P. hispidus*, and incubated for 1 month.

The fungus attacked the shavings readily, but mycelial growth did not appear to be quite so vigorous as on untreated shavings exposed to the same conditions. At the end of 1 month the shavings were removed, dried, and each batch extracted with boiling alcohol for 10 minutes. The extracts were tested in the usual way with phloroglucin and hydrochloric acid, the whole seven, both from conifers and hard woods, giving a pink

coloration. Treated shavings, kept moist for a smilar period, but not exposed to the action of the fungus, gave uniformly colourless results. Hence Czapek's hadromal reaction is not a suitable means of distinguishing between sound and decayed woods, since sound wood contains enough hadromal to give a very distinct reaction.

This hadromal contained in sound wood renders the method valueless for the purpose of testing for ligninase, the pink coloration of the alcoholic extract being given both before and after exposure to the fungus. The wood being treated in some such way as that described above, in order to remove the hadromal already present, the reaction becomes a valid test for ligninase.

In this way it is shown that the mycelium of *Polyporus hispidus* contains a ligninase capable of attacking the lignified tissues of the wood.

Hemicellulase. As the hemicelluloses are often a constituent part of the cell-wall, amounting, on the average, to from 9 to 12 per cent. of the dry weight of wood, it is therefore possible that they may play a part in the nutrition of a wood-destroying fungus.

Schellenberg has differentiated between those enzymes which resolve cellulose, and those which attack the hemicelluloses, and has also shown that some of the hemicellulases are specific in their activities. The hemicelluloses are more easily hydrolysed than the true celluloses, and also differ in their decomposition products. While the celluloses are hydrolysable to glucose, the hemicelluloses yield other sugars. The products of hydrolysis are sometimes pure sugars, and sometimes a mixture, the chief sugars to be obtained being mannose, dextrose, galactose, xylose, and arabinose. The nomenclature of the hemicelluloses is based on the sugars given on reduction.

The hemicellulose used in these experiments was paragalactan, which forms the greater part of the endosperm of the date (*Phoenix dactylifera*).

Date stones were thoroughly washed, and their outer coats removed by grinding on a small emery wheel. They were then cracked open, the embryos cut out, and the remaining endosperm autoclaved in order to deactivate any enzymes present. They were kept for future use in distilled water to which a trace of toluene had been added. Very thin slices of the paragalactan prepared as above were cut on a microtome, and small pieces of these were suspended in hanging drops in Van Tieghem cells.

Enzyme suspension No. 1 was used in these cells, and two sets of controls were used. The first was distilled water, while the second was the enzyme suspension after it had been deactivated by autoclaving.

A small quantity of toluene was added to all the cells as an antiseptic. Erosion started after 1 month, but was not very noticeable. After 2 months distinct erosion was seen in all the cells with the enzyme. In no case was the destruction of the endosperm so marked as in the similar pieces described by Zeller. There was no erosion at all in any of the controls.

All the drops remaining after the two months were tested for the presence of bacteria, but were found to be free from contamination.

In order to confirm these results some of the endosperms were ground to a fine meal on a rasp, and about 0·3 gm. placed in each of twelve test-tubes. These were divided into three batches.

1. Hemicellulose + Enzyme No. 1 + Toluene
2. ,, + Enzyme No. 2 + ,,
3. ,, + Enzyme No. 1 (autoclaved) + ,,
4. ,, + Distilled water + ,,

They were all incubated at 25° C. for 14 days. The liquid was then filtered off, and tested for reducing sugars with Fehling's solution. Batches No. 1 and No. 2 gave strongly positive results, while the controls gave clear solutions.

Hence it is clear that the fungus *Polyporus hispidus* contains hemicellulase, capable of hydrolysing the hemicellulose paragalactan to galactose and arabinose.

Oxidase. The presence of oxidase was tested for, using a solution of guaiacum as an indicator.

Enzyme Suspension No. 1. To 3 c.c. of the extract in a test-tube was added three drops of hydrogen peroxide and five drops of a 1 per cent. solution of guaiacum. A blue coloration was produced in 4 minutes.

Enzyme Extract No. 2. To 1 c.c. of extract was added 3 c.c. of distilled water, four drops of hydrogen peroxide and five drops of guaiacum. A blue colour was produced in $3\frac{1}{2}$ minutes.

Distilled water and boiled enzyme extracts gave no coloration at all. Hence it is concluded that there is a considerable quantity of oxidase in the mycelium of *Polyporus hispidus*.

Catalase. Catalase was tested for by adding the enzyme extract to hydrogen peroxide.

Extract No. 1. When added to hydrogen peroxide some effervescence took place, showing that oxygen was being liberated.

Extract No. 2. When added to hydrogen peroxide a great deal of frothing occurred, so much that the test-tube was filled with the foam produced by about 1 c.c. of extract and 1 c.c. of peroxide.

Distilled water and boiled extract produced no frothing, showing that the mycelium of *P. hispidus* contains an oxidase.

Summary.

The characteristics of the *Polyporus hispidus*, when grown on artificial media, both solid and liquid, are described and compared with those given by Baxter.

Growth on wood of ash under laboratory conditions produces a rot which is indistinguishable from that occurring naturally. The distribution of the hyphae in the wood is described.

The mode of penetration of the cell-wall by the hyphae is figured. It is apparently by pits in the early stages of decay, but by bore-holes, formed entirely by enzyme activity, in the more advanced rot.

The rate of growth of the fungus under controlled conditions has been measured, and shown to be 0·5 cm. per month.

Successful inoculation experiments have been carried out with young trees, confirming the results of Baxter, who states that the fungus can attack young, living sap wood.

An investigation of the enzymes produced by the mycelium has been carried out, and a method evolved for demonstrating the presence of a ligninase. This is a modification of Czapek's "hadromal" reaction.

The following enzymes are shown to be present in the mycelium: emulsin, diastase, invertase, ligninase, hemicellulase, oxidase, and catalase. The list is not intended to be exhaustive.

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EXPLANATION OF PLATES VI—VIII.

PLATE I.

Fig. 1. Camera lucida drawing of ash wood after 4 months' attack by *Polyporus hispidus*. Note that the penetration of the cell-wall is almost entirely through the pits.

PLATE II.

Fig. 2. Camera lucida drawing of ash wood after 6 months' attack by *Polyporus hispidus*. The shaded cells to the left of the drawing are full of large empty brown hyphae, and form the edge of the zone line. Note the penetration by bore-holes, as in Text-fig. 2, and the large dead hyphae scattered about in the vessels.

Fig. 3. Drawing of surface of wood attacked by *Polyporus hispidus*, under the ring illuminator. The mycelium in the vessels is drawn with the camera lucida, while the wood is semi-diagrammatic. Note the considerable development of mycelium in the vessels; this would be washed out if sections were cut.

PLATE III.

Fig. 4. Section of trunk of 85-year-old ash tree attacked by *Polyporus hispidus*. The rot produced can be seen in the centre. In this case the probable path of entry of the fungus was by the branch stub shown.

Fig. 5. "Bore-hole" of *Polyporus hispidus* in ash. × 1200.

(*Received June 2nd*, 1928.)

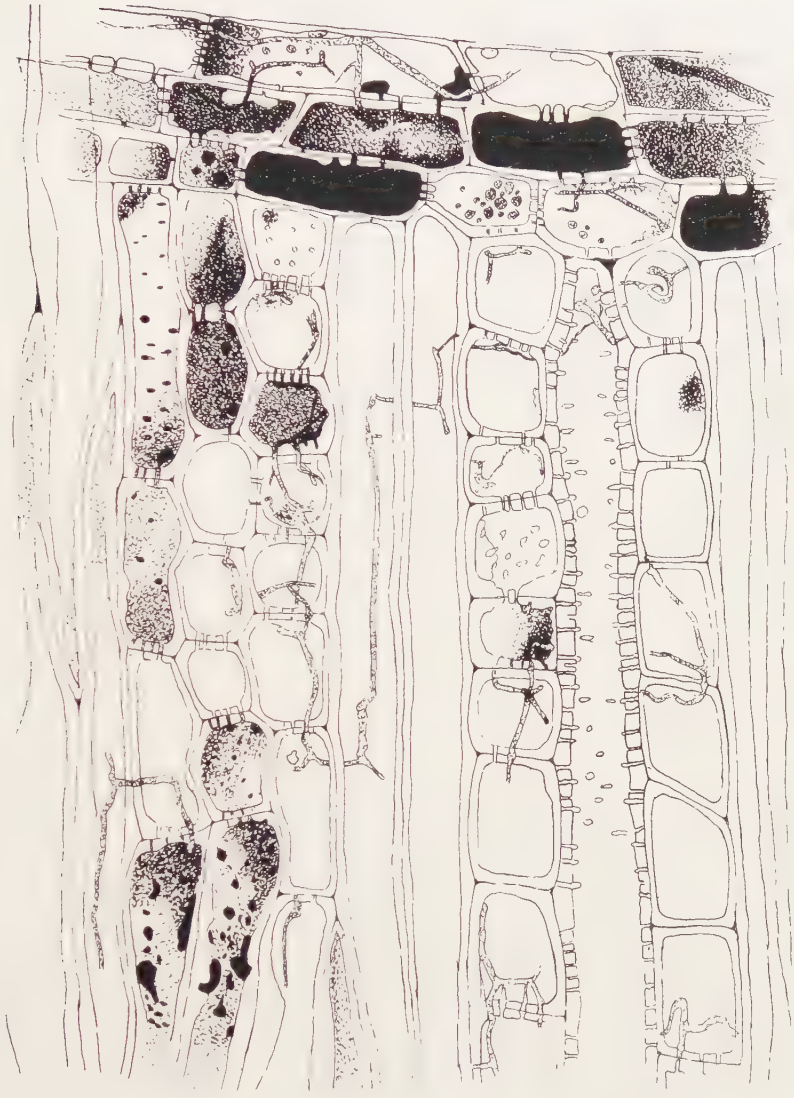


Fig. 1.

NUTMAN.—Studies of Wood-destroying Fungi (pp. 40–64).

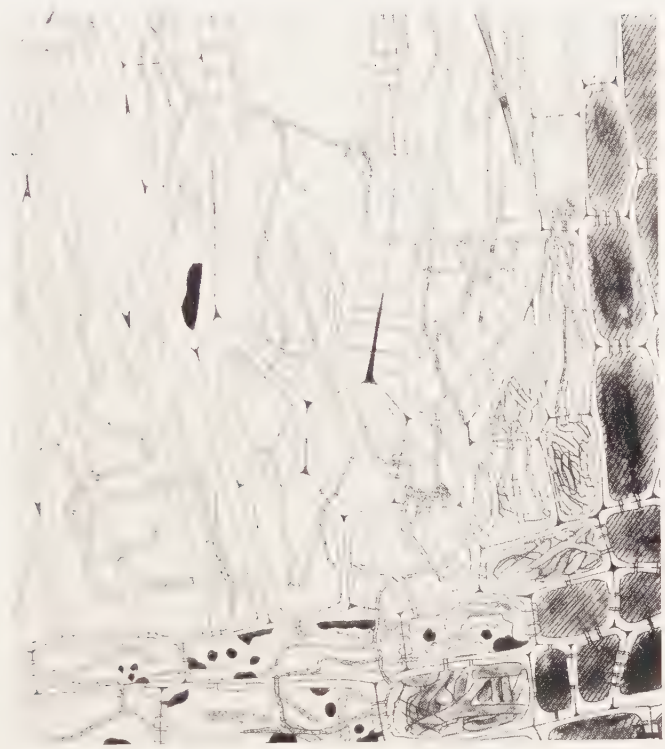


Fig. 2.

Fig. 3.

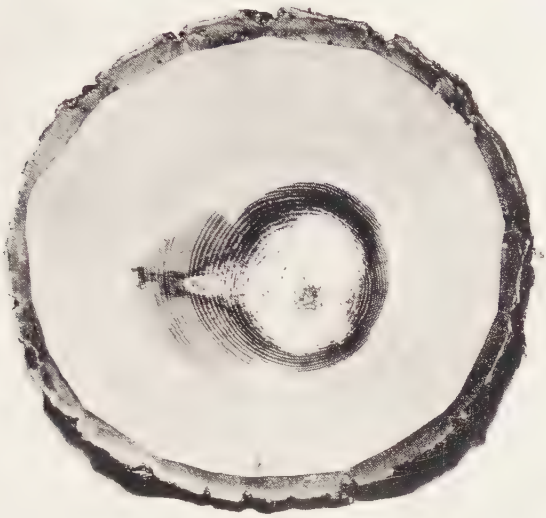


Fig. 4.

Fig. 5.

NUTMAN.—Studies of Wood-destroying Fungi (pp. 40–64).

THE BIOLOGY OF OAT SMUTS

II. VARIETAL RESISTANCE

By KATHLEEN SAMPSON, M.Sc. (Lond.).
(*University College of Wales, Aberystwyth.*)

(With Plate IX and 1 Text-figure.)

CONTENTS.

I. Introduction.

Proof of the existence of biological specialisation in loose and covered smuts of oats was given by Reed (9) in 1924, and by Sampson (13) in the following year. Each writer worked with the same races, and the results obtained on a number of oat varieties in Missouri and at Aberystwyth showed substantial agreement, leaving no doubt as to the validity of the distinctions drawn between the four races under study.

In 1927 Reed (10) published data which gave further evidence of biological specialisation in oat smuts. He worked with collections of chlamydospores of *Ustilago avenae*, which differed from forms previously studied in their capacity for attacking varieties of the species *Avena sterilis*. Reed found that the collections belonged to two distinct biological species, the

one capable of attacking selections of Fulghum but giving no infection on Red Rustproof, while the second gave exactly contrary results, attacking Red Rustproof but not Fulghum.

There are therefore at present four well-defined biological species of *U. avenae* and two of *U. levis*. They can be distinguished by suitable varieties or selections of the hosts indicated in the list below.

Country of origin	Susceptible	Resistant
U. avenae—		
1. Wales	*A. sativa*, Potato, Radnorshire sprig, and others	*A. strigosa, A. nuda, A. sterilis*
2. U.S.A.	*A. sativa, A. strigosa, A. nuda*	*A. sterilis*
3. U.S.A.	*A. sterilis*, Fulghum	*A. sterilis*, Red Rustproof
4. U.S.A.	*A. sterilis*, Red Rustproof	*A. sterilis*, Fulghum
U. levis—		
1. Wales	*A. strigosa, A. brevis*	*A. sativa, A. nuda, A. sterilis*
2. U.S.A.	*A. sativa, A. strigosa, A. nuda*	*A. brevis, A. sterilis*

During the past three years it has been possible to study in Wales the infection capacities of certain other spore collections of both species of oat smut. The results indicate that two more biological species must be added to the above list. The first is a race of *U. avenae* (L 11)[1] which has been found capable of attacking only varieties of *A. brevis* and *A. strigosa*, from which species it was originally isolated. The second is a race of *U. levis* (C 3), which was obtained from *A. sativa*, Grey Winter in England. This race shows a decided similarity to the Missouri strain of *U. levis* (C 2) first studied by Reed (9), but it differs from the latter in that it gives low or negative results on *A. strigosa orcadensis* (Cc 521) and on *A. nuda* (Cc 2495), whereas these varieties are highly susceptible to the Missouri strain. The experimental data are discussed below.

With the object of obtaining data relating to the percentage attack on commercial varieties, over two hundred samples of oats from different districts in the British Isles were grown at the Station in 1927. The results are interesting in connection with the general problems of varietal resistance and distribution of biological species. Not a single sample of *A. sativa* showed any infection from *U. levis*, a result which confirms the previously expressed belief as to the rare occurrence in Britain of strains of this species capable of attacking *sativa* varieties (13). The results also agree with those of Stapledon (16) which indicated that in this country

[1] References in brackets indicate the index numbers given by the author to the collections of spores under study. The letters L and C denote the species *U. avenae* and *U. levis* respectively. Numerals refer to particular strains, and small letters have been used to distinguish collections of spores harvested on different dates or by different methods.

infection is usually heaviest on the older varieties such as are included in the Winter, Potato and Sprig groups[1]. It is interesting to find that the same varieties were among the most susceptible of those tested experimentally with the Welsh strain *U. avenae* (L 1) isolated originally from Potato oats. It is probable that this represents a biological species widely distributed in the British Isles.

A microscopic examination was made of the pales of grain from samples which produced a relatively high percentage of infection in the field. From this it was evident that "flowering infection" as defined by Zade (19) was of general occurrence. It follows that the extent to which oat varieties open their pales and the atmospheric conditions during and shortly after the flowering period, must be counted among the important factors which determine the intensity of the smut attack in the subsequent crop. It is not improbable that a variety which gives high infection figures when tested under special experimental conditions will not necessarily fall among the most susceptible varieties when natural infection is in question. It is possible that some of the newer varieties are largely "smut escaping" though not strongly "smut resistant."

Table I.

Showing the incidence of smut on shelled and hulled grain of certain oat varieties. Sown March 13th, 1925. *Farm cage. Reference C* 138, *I.*

		Grain in husk				Grain shelled			
Variety	Station number	Number of plants	Smutted plants %	Number of panicles	Smutted panicles %	Number of plants	Smutted plants %	Number of panicles	Smutted panicles %
I. Loose smut ex *A. sativa* (L 1)—									
A. strigosa glabrescens	363	210	0	316	0	202	0	308	0
A. sativa, Black Bell	2475	119	0	144	0	131	0	165	0
„ Orion	2477	135	0	150	0	144	0	200	0
„ Record	1642	165	0	232	0	99	7·1	150	6·0
„ Ceirch du bach	1080	182	0·6	252	0·4	175	12·6	287	10·8
„ Potato	1029	161	1·9	190	3·2	51	45·1	81	37·0
II. Covered smut ex *A. strigosa* (C 1)—									
A. strigosa pilosa	362	207	5·8	260	5·4	200	51·0	360	58·3
„ *glabrescens*	363	179	5·0	229	5·2	154	47·4	185	36·8
A. brevis	1614	200	0·5	342	0·3	68	2·9	133	1·5
A. sativa, Record	1642	169	0	214	0	110	0	147	0
„ Ceirch du bach	1080	196	0	259	0	181	0	275	0

[1] Here and elsewhere in the paper the grouping of varieties follows the classification given by Marquand (6).

II. Methods of testing oat varieties for resistance to smut.

Resistance tests carried out during the seasons 1922 to 1924 with heavily contaminated grain sown under field conditions gave on the whole remarkably low infection, and it became necessary to modify the technique adopted in these early experiments (13). The different methods which have been tested are described below. The spore material was obtained from previous experiments and it included four of the biological species which have been already defined with reference to their infection capacities on certain oat varieties.

1. *Shelled grain* versus *grain in husk.*

In oats and barley (5, 17, 3) the chances of infection are considerably increased by removing the pales and applying chlamydospores to the surface of the caryopsis[1]. Some results obtained by sowing in the open shelled and hulled grain of certain oat varieties contaminated with two biological species of *U. avenae* and *U. levis* are summarised in Table I. The increase due to removal of the husk was in the case of *U. avenae* on Potato as much as 33·8 per cent., and in the case of *U. levis* on *A. strigosa pilosa* 42·9 per cent. It is important to notice that the use of shelled grain emphasises the differences between the biological species; susceptible varieties show considerably increased infection, but resistant varieties continue to give negative results. Johnston (5) arrived at a somewhat similar conclusion.

Further evidence of the distinct advantage of shelled grain in experimental work of this kind is given in Tables III, IV and VII. In later experiments (Tables V and VI) only shelled grain was used.

2. *Influence of different dates of sowing under field conditions.*

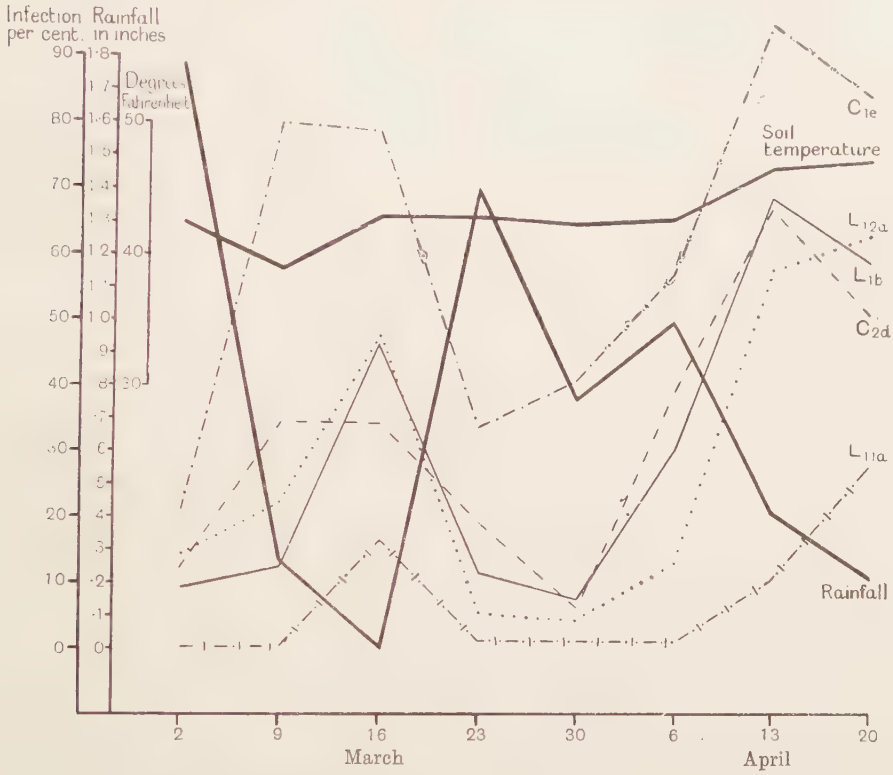
Several investigators (2, 5, 11, 18) have shown that the intensity of the attack of smut fungi in oats is influenced considerably by the temperature and by the moisture content of the soil during the period of germination. All agree that relatively dry soil and a moderately high temperature favour infection.

In 1927, starting on February 28th, eight sowings were made at weekly intervals of shelled grain contaminated with five biological species of smut. Suitable susceptible varieties of oats were used as hosts for the different races of smut. Each sample was sown in duplicate at the rate

[1] The method of obtaining chlamydospores free from fragments of the host plant is described in an earlier paper (14).

of 150 grains per 5-foot row. The ground was uniform and the experiment covered only a small area.

The percentage infection of the different lots is represented graphically in Fig. 1, together with the average temperature of the soil for the



Rainfall in inches. Average of week, 2 days before to 5 days after sowing.

Soil temperature at 9 a.m. Depth 4 inches. Average of week after sowing.

— • | • — • | • — *U. avenae* L 11*a* on *A. strigosa glabrescens.* 1706 Ce 5.

— — — — — — *U. avenae* L 1*b* on *A. sativa.* Potato 3191.

. . . . . *U. avenae* L 12*b* on *A. sativa.* Potato 3191.

— • — • — • — • *U. levis* C 1*e* on *A. strigosa orcadensis.* 3190.

— — — — — *U. levis* C 2*d* on *A. sativa.* Potato 3191.

Fig. 1. Graph showing the percentage infection from weekly sowings of oats contaminated with five spore-collections of loose and covered smut. Shelled grain. Phenological Garden, 1927.

week following each date of sowing, taken at a depth of 4 inches. The total rain which fell during the interval, 2 days before to 5 days after sowing, is also shown in the graph. This may be taken as a general

indication of the changes which occurred in the moisture content of the soil during the experimental period.

The intensity of attack produced by the different biological species showed a wide range of variation. *U. levis* (C 1) varied between the limits 34 to 95 per cent. on the different dates of sowing. This high infection is probably due in part to the heavy contamination of the grain and to the good viability of the spores. *U. avenae* (L 11) gave on the other hand low infection, the figures ranging from 0 to 28 per cent. This was probably caused by the poor viability of the spores which gave a germination of only 2 per cent., and by the fact that a smaller bulk of material was available for contamination (Fig. 1).

The two species, *U. avenae* and *U. levis*, showed on the whole the same general response to changes in the conditions which operated on the grain which was sown on different dates. The graphs for the five individual samples show two peaks which appear to be correlated with two periods of low rainfall. The temperature of the soil did not vary widely during the first seven weeks of the experimental period, and rainfall rather than temperature appears to have been in this experiment the critical factor in determining the relative intensity of the attack. That a relatively low moisture content of the soil favours infection of oats by both species of smut has been shown by others who have made experiments under artificial (2, 11, 18) or natural conditions (5). The present experiment supports this view.

3. *Infection produced under controlled conditions of temperature and moisture.*

The data discussed in the previous section explain the uncertainty of resistance trials conducted under conditions which are subject to vagaries of the weather. It is clearly important that both temperature and humidity should be under control when the grain germinates.

A preliminary trial in 1924 showed that it is only necessary to control these factors for a short period, and the following method was adopted in 1925 and in subsequent seasons. Certain details of technique were suggested by the work of Reed (9).

Germination on sand (*Method C*). Uniform, dry and sterilised sand was mixed with sufficient water to give a moisture content equal to 20 per cent. of its saturation capacity[1]. Soaked earthenware saucers, glazed on the inside, were partly filled with sand. Shelled grain coated with spores was sown on the smoothed out surface and covered uniformly

[1] The sand used passed through a 1·0 mm. sieve, and had a *p*H value of 8·2.

with sand to a depth of 1 in. The dishes were placed in an incubator at 22° C. together with open dishes of water. The small quantity of water lost by the sand was replaced daily by spraying the surface until the dish regained its original weight. In most varieties the majority of the seedlings were visible above the surface by the fourth or fifth day. Each was marked by a small rubber ring and the dishes were placed on the greenhouse bench. Finally, marked plants were transferred to large pots or to boxes of soil in which they were allowed to mature.

In Table II the results are given of trials with four biological species. In series *a* and *b* the seedlings were planted in duplicate Doulton pots;

Table II.

Showing the infection of shelled grain germinated in sand under controlled conditions of temperature and moisture. Two biological species of U. avenae *and* U. levis. 1925. *Reference C* 86.

Species and variety of host	Ref. to series	Number of plants	Smutted plants %	Number of panicles	Smutted panicles %	Number of plants	Smutted plants %	Number of panicles	Smutted panicles %
		U. avenae ex Wales (L 1)				*U. avenae* ex U.S.A. (L 2)			
A. nuda (2495)	*a*	8	12·5	22	4·5	6	100	24	100
	b	12	0	20	0	8	100	25	100
	c	11	0	28	0	3	100	8	100
	Average		*4·2*		*1·5*		*100*		*100*
A. sativa, Sandy (2448)	*a*	9	0	32	0	9	11·1	39	7·7
	b	9	0	35	0	9	33·4	36	38·9
	c	8	0	26	0	8	25·0	24	16·7
	Average		*0*		*0*		*23·2*		*21·1*
A. strigosa orcadensis (2662)	*a*	10	0	71	0	8	50·0	80	45·0*
	b	11	0	70	0	9	88·9	75	76·0*
	c	8	0	36	0	5	80·0	28	78·6*
	Average		*0*		*0*		*73·0*		*66·5*
		U. levis ex Wales (C 1)				*U. levis* ex U.S.A. (C 2)			
A. sativa, Potato (1029)	*a*	9	0	32	0	7	28·6	27	25·9†
	b	10	0	32	0	8	50·0	23	56·5
	Average		*0*		*0*		*39·3*		*41·2*
A. strigosa orcadensis (2662)	*a*	9	100	56	100	10	80·0	63	68·2
	b	11	100	60	100	10	70·0	76	59·2
	c	4	75	13	69·2	8	62·5	34	55·8
	Average		*91·7*		*89·8*		*70·8*		*61·1*

* On this host the biological species L 2 developed slowly, infecting the glumes and pales only to a slight extent and retarding growth comparatively little (Pl IX, fig. 1 (*d*)).

† One plant showed sori of chlamydospores on the flag-leaves of four tillers.

in series *c* large wooden boxes were used. The data for each series are given separately in order to show that the method gives concordant results with relatively few plants per unit. In later tests by this method the results given represent the average from two pots each of which contained 9 to 11 plants (Tables V and VI).

Plate IX, fig. 1, shows the appearance of four pots of *A. strigosa* infected by four distinct biological species of smut. The races differ not only in the number of plants with smutted panicles (Table II) but also in their influence on the growth of the host plant. *U. levis* (C 1), which gave 100 per cent. infection, also caused the greatest reduction in height (pot *a*).

This method was equally satisfactory in 1926 and 1927, duplicate pots giving close agreement.

Germination on filter-paper. In 1927 certain samples of grain, shelled and shaken with chlamydospores, were sown in rows on double sheets of damp filter-paper, which were rolled up and placed on the shelf of an incubator running at 22° C. After 3 days the germinated seedlings were planted in moist soil in boxes and left until maturity. The results obtained with fourteen samples (Tables V and VI) agreed closely with those from parallel tests in sand. Further trials of the method are in progress.

4. *Infection caused by contaminating the open flowers.*

In 1925 an attempt was made to test the resistance of a number of varieties to selected strains of smut, by placing chlamydospores between the pales when the plants were in flower, a method which was recommended by Zade (19) as giving higher infection than that which usually followed the sowing of contaminated grain in spring. Preliminary observations showed that oat varieties varied widely in the extent to which their pales opened, and in order to test a large number of varieties the spores were placed between pales, temporarily separated by means of forceps. The danger of accidental contamination by air-borne spores was minimised by enclosing the panicles shortly after emergence in pollen-proof bags, and opening these only for the purpose of applying the spores. Grain from panicles enclosed but not inoculated gave, with one single exception, healthy plants in the following year.

From series A and B (Table III) spikelets were cut from time to time, fixed in 70 per cent. alcohol and examined microscopically. In some spikelets germinated spores were found on the second day after inoculation. In others, cut 2 to 9 days after inoculation, no sign of germination

was evident. The following figures indicate the number of spikelets in which germination had occurred 2 to 14 days after inoculation. Twenty spikelets were examined in L 1, C 1 and C 2, nineteen in the case of L 2. The host was *A. sativa*, Potato. *U. avenae* L 1, 20; *U. avenae* L 2, 17; *U. levis* C 1, 11; *U. levis* C 2, 20.

Germination counts were not possible, but it was evident that a relatively small number of the spores introduced between the pales had germinated.

Pales from the ripe grain were also examined microscopically at a later date. The grains were soaked in water for 12 hours and then fixed in alcohol. The pales were treated by the lacto-phenol and cotton blue method and mounted so that the inner surface of each pale could be examined.

Twenty-one grains from series C were examined by this method and thirteen showed mycelium or gemmae such as Zade(19,20) described (Plate IX, fig. 2 (*c*)). In some cases it was possible to trace a connection between the mycelium and germinated spores of *Ustilago*. It was evident that germination between the pales occurred in both species of smut and on both susceptible and resistant varieties.

Grain contaminated by the above method was sown in boxes in an unheated greenhouse on March 11th, 1926. Later the plants were spaced out in rows on the farm cage. Parallel tests were made with grain shelled and grain in the husk, which was shaken with spores which had been kept in store during the winter. The results for each series are given in Table III.

It should be stated in the first place that the positive results which the method of "flowering infection" gave in A I and B I are entirely in harmony with the infection capacities of the particular biological species under test, as determined by previous trials. The spore collection, L 10, isolated from Grey Winter, was then tested for the first time. It appears to resemble *U. avenae* L 1, isolated from Potato, but further work with this collection was not carried out.

Comparing the three methods of treatment, it is necessary to consider separately the series A, B and C. With two races of *U. levis* (series B), grain contaminated in spring gave higher infection than that obtained from flowers dusted with spores in the previous summer (Method I).

In series A, method I is sometimes superior, sometimes inferior to method II, the contamination of shelled grain. In series C, which involved a different collection of spores, method II gave completely negative results with the exception of a single infected plant of Earl Haig. Method I

Table III.

Showing the infection of certain oat varieties by three methods of contamination with chlamydospores of U. avenae *and* U. levis. 1925–26. *Reference C* 162 *and C* 148.

Species and variety of host	Ref.	I. Flowering period			II. Shelled grain			III. Grain in husk		
		No. of plants	No. of plants smutted	Smutted plants %	No. of plants	No. of plants smutted	Smutted plants %	No. of plants	No. of plants smutted	Smutted plants %
A. *U. avenae*—										
A. sativa, Potato	L 1	38	10	26	20	0	0	20	0	0
,, ,,	L 2	50	9	18	17	6	35	24	1	4
,, Sandy	L 1	26	0	0	17	0	0	15	0	0
,, ,,	L 2	27	15	56	21	11	52	21	9	43
A. strigosa	L 1	17	0	0	10	0	0	18	0	0
,,	L 2	10	0	0	19	0	0	17	0	0
A. nuda	L 1	43	0	0	17	0	0	—	—	—
,,	L 2	37	4	11	20	7	35	—	—	—
B. *U. levis*—										
A. sativa, Potato	C 1	55	0	0	22	0	0	23	0	0
,, ,,	C 2	51	6	12	21	7	33	21		19
A. strigosa	C 1	17	11	65	14	14	100	17	13	78
,,	C 2	11	0	0	15	3	20	16	4	25
C. *U. avenae*—										
A. sativa, Ceirch du bach*	L 10	47	10	21	21	0	0	19	0	0
,, Potato*	,,	35	4	11	18	0	0	18	0	0
,, Potato	,,	14	5	35	—	—	—	—	—	—
,, Radnorshire sprig*	,,	17	2	12	21	0	0	18	0	0
,, Grey Winter*	,,	7	1	14	17	0	0	18	0	0
,, Earl Haig*	,,	10	1	10	15	1	7	19	0	0
,, Earl Haig	,,	10	1	10	—	—	—	—	—	—
,, Black Tartar	,,	31	1	3	19	0	0	14	0	0
,, Marvellous	,,	24	1	4	—	—	—	—	—	—
,, Record	,,	19	0	0	16	0	0	19	0	0
,, Gelbhafer*	,,	21	0	0	20	0	0	20	0	0
,, Markton*	,,	2	0	0	19	0	0	23	0	0
,, Captain	,,	15	0	0	17	0	0	12	0	0
,, Golden Rain	,,	8	0	0	21	0	0	15	0	0
,, Waverley	,,	27	0	0	15	0	0	15	0	0
,, Crown	,,	18	0	0	20	0	0	19	0	0
,, Leader	,,	24	0	0	21	0	0	19	0	0
,, Abundance	,,	19	0	0	18	0	0	21	0	0
,, King	,,	18	0	0	20	0	0	20	0	0
,, Victory	,,	16	0	0	18	0	0	15	0	0
*A. strigosa orcadensis**	,,	61	0	0	20	0	0	19	0	0
*A. brevis**	,,	24	0	0	21	0	0	18	0	0

* Contaminated by dusting *exserted* stigmas with chlamydospores. In other cases the spores were placed between the pales, which were artificially separated with forceps.

gave positive results in nine varieties, but in no case was the infection severe, the highest figure being 35 per cent. on Potato.

It is not improbable that the above differences are related to the viability of the chlamydospores. It has been shown in a previous paper (14) that the spores of *U. levis* remain viable in store for a considerable period, whereas chlamydospores of *U. avenae*, particularly when collected from immature plants, rapidly lose their power of germination. In the samples under discussion collections L 1 and L 2 gave low but appreciable germination in spring 1926, while L 10 lost all trace of viability as early as October 1925. Positive results were not to be expected from grain dusted with spores of this collection in March 1926. It is probable therefore that plants infected by L 10 (method I) owed their infection to mycelium or gemmae formed on the pales in the previous summer. Infection in series A and B (method I) may have been due either to such resting mycelium or to the germination of those chlamydospores which had retained their viability during the period of storage.

The method of contaminating flowers was distinctly laborious, and the results obtained are clearly less satisfactory than those given by shelled grain sown on sand as described in the previous section.

Arland (1), Diehl (4) and Rosch (12) have tested the resistance of German varieties of oats by introducing chlamydospores already germinated, between the pales of ripe grain. High percentages of infection were seldom obtained, though the method was considered to be more reliable than the dusting of grain in husk.

III. Further biological species of *U. avenae* and *U. levis*.

The essential differences between the biological species L 1 and L 2 of *U. avenae*, and C 1 and C 2 of *U. levis*, have been clearly brought out in the experiments designed to test different methods of technique. The present section deals with four additional collections of spores, two of which possess infection capacities distinct from any biological species previously identified.

1. *U. avenae* (L 11) isolated from *A. strigosa glabrescens* in Wales.

(*a*) *Field experiment.* 1926. *Reference C* 166.

Thirty-two samples of oats were contaminated in March 1926 with chlamydospores of L 11 and L 12, two collections of *U. avenae* obtained from *A. strigosa glabrescens* and *A. sativa*, Potato respectively. With the exception of the varieties of Black Mesdag and Hull-less both shelled and hulled grain of each variety were tested. Each sample was sown in

Table IV.

Showing the behaviour of two biological species of Ustilago avenae *on thirty-one varieties of oats.*
Sown March 27th, 1926. Upper Ridge Field. Reference C 166.

Station number	Variety	*Ustilago avenae* ex *Avena strigosa* Reference L 11						*Ustilago avenae* ex *Avena sativa* var: Potato Reference L 12					
		Shelled grain			Grain in husk			Shelled grain			Grain in husk		
		Total no. of panicles	No. of smutted panicles	Smutted panicles %	Total no. of panicles	No. of smutted panicles	Smutted panicles %	Total no. of panicles	No. of smutted panicles	Smutted panicles %	Total no. of panicles	No. of smutted panicles	Smutted panicles %
362	*A. strigosa pilosa*	221	189	93	244	131	53	152	0	0	196	0	0
521	,, *orcadensis*	185	167	90	181	83	46	92	0	0	147	0	0
2877	,, *glabrescens*	131	105	80	140	69	49	138	0	0	134	0	0
2384	*A. brevis*	117	78	66	183	114	62	180	0	0	162	0	0
2855	*A. sativa*, Potato	136	0	0	132	0	0	197	174	88	217	75	35
2798	,, Captain	166	0	0	123	0	0	145	56	39	168	6	4
975	,, Ceirch du bach	201	0	0	162	0	0	156	57	36	259	24	9
1103	,, Radnorshire sprig	181	0	0	176	1	0·6	162	42	26	151	26	17
2799	,, Bountiful	156	0	0	143	0	0	123	34	26	192	20	10
2854	,, Tartar King	109	0	0	155	0	0	140	36	26	139	3	2
2860	,, Grey Winter	152	0	0	172	0	0	134	19	14	193	3	2
2804	,, Record	107	0	0	151	1	0·7	176	23	13	173	15	9
2810	,, Yielder	143	0	0	124	0	0	121	11	9	151	9	6
2801	,, Waverley	157	0	0	149	0	0	165	12	7	194	5	3
2806	,, Black Tartar	156	0	0	121	0	0	132	6	5	127	2	2
2797	,, Superb	124	0	0	135	0	0	132	3	2	184	0	0
2802	,, Haig	135	0	0	170	0	0	165	3	2	168	1	0·6
2809	,, Marvellous	132	0	0	142	0	0	138	1	0·7	173	0	0
2805	,, Victory	145	0	0	125	0	0	174	1	0·6	192	0	0
2782	,, Gelbhafer	159	0	0	187	0	0	163	0	0	217	0	0
2791	,, Markton	136	0	0	164	0	0	134	0	0	183	0	0
2792	,, King	175	0	0	159	0	0	168	0	0	192	0	0
2794	,, Crown	151	0	0	155	0	0	130	0	0	192	0	0
2795	,, Golden Rain	139	0	0	156	0	0	145	0	0	209	0	0
2796	,, Ligowo	163	0	0	171	0	0	162	0	0	204	0	0
2800	,, Leader	152	0	0	162	0	0	176	0	0	178	0	0
2807	,, Abundance	148	0	0	178	1	0·6	210	0	0	183	0	0
2371	,, Black Mesdag	170	1	0·6	—	—	—	168	0	0	—	—	—
1610	,, Black Mesdag	138	0	0	—	—	—	139	0	0	—	—	—
2803	,, Supreme	124	0	0	129	0	0	141	1	0·7	158	0	0
2808	,, Goldfinder	138	0	0	135	0	0	188	0	0	159	0	0
2495	*A. nuda*, Hull-less	151	0	0	—	—	—	155	0	0	—	—	—

5-feet drills at the rate of 200 grains per row. The intensity of the attack of smut was estimated at maturity by counting the healthy and infected panicles in each row. The results are summarised in Table IV.

The difference between the two races of smut was very striking, especially in the series which included shelled grain. *U. avenae* (L 11), isolated from *A. strigosa*, infected only the three sub-species of *A. strigosa* together with *A. brevis*. *A. nuda* and twenty-six varieties of *A. sativa* remained immune. The intensity of the attack varied from 66 to 93 per cent. *U. avenae* (L 12), isolated from *A. sativa*, Potato, failed to infect *A. nuda*, *A. brevis* and *A. strigosa*, but thirteen varieties of *A. sativa* were attacked, the degree of infection ranging from 2 to 88 per cent.

These results include the highest infection figures yet obtained in Wales under field conditions, and it is interesting to find that the grain was subject to meteorological conditions which have proved to be favourable for infection of oats by smut. March 1926 was exceptionally dry with a total rainfall of only 1·16 in. The rain which fell during the interval, 2 days before to 5 days after sowing, amounted only to 0·177 in. The average temperature at a depth of 4 in. in the soil at 9 a.m. daily was 42·9° F. The data are of interest in connection with Fig. 1.

Table V.

Showing the behaviour of four spore collections of U. avenae *on twelve varieties of oats.* 1927. *Reference C* 166.

Species and variety of host	Percentage infected plants produced by spore collections of *U. avenae*: L 1, Wales ex *A. sativa*	L 12, Wales ex *A. sativa*	L 2, U.S.A.	L 11, Wales ex *A. strigosa*
Season 1927. Germinated in sand—				
A. sativa, Sandy (1799)	100	90	100	0
,, Potato (2855)	100	100	100	0
,, Radnorshire sprig (1103)	80	30	82	0
,, Victor (2394)	90	40	100	0
,, Abundance (2807)	0	0	100	0
,, Record (2804)	10	30	100	0
,, Black Tartar (2806)	90	20	100	0
,, Markton (2375)	0	0	0	0
A. nuda (2495)	0	0	100	0
A. strigosa orcadensis (521)	0	0	30	67
A. strigosa glabrescens (2877)	0	0	0	30
A. brevis (2384)	0	0	0	10
Season 1927. Germinated on filter-paper—				
A. sativa, Potato (2855)	100	100	100	0
A. nuda (2495)	0	—	100	—
A. strigosa orcadensis (521)	—	0	—	100

(*b*) *Germination in sand under controlled conditions of temperature and moisture.* 1927.

The same races of *U. avenae*, L 11 and L 12, were tested again in 1927 on twelve varieties of oats by the method of germination in sand (p. 70). Two additional samples of *U. avenae*, L 1 and L 2, were also included for comparison. The results are summarised in Table V.

U. avenae (L 11) again infected only varieties of *A. brevis* and *A. strigosa*. *U. avenae* (L 2, Missouri) infected *A. nuda* and a number of *sativa* varieties heavily and produced some infection on *A. strigosa orcadensis*. Collections L 1 and L 12 probably represent the same biological species. They infected certain *sativa* varieties but gave negative results on *A. nuda*, *A. brevis* and *A. strigosa*, and by these characters are distinguished from L 2 and L 11. It is evident that at least three biological species of *U. avenae* are represented by these collections[1].

2. *U. levis* (C 3) isolated from *A. sativa*, Grey Winter in England.

In 1925, by the kindness of Dr G. Pethybridge, two collections of *U. levis* were received from Hertfordshire, the one (C 3) on a *sativa* variety Grey Winter, the other (C 4) on an unknown variety. Infection experiments conducted with each collection indicate that both represent the same biological species.

Table VI shows the results obtained in 1926 and in 1927 with four spore collections of *U. levis*. The tests were carried out by method C.

U. levis (C 1) produced infection only on varieties of *A. strigosa* and *A. brevis*, while *U. levis* (C 2) attacked varieties of *A. strigosa*, *A. nuda* and *A. sativa*. These results were expected from previous experience with the collections.

Collections C 3 and C 4 produced an intense attack on three *sativa* varieties, including the original host Grey Winter, but they gave low or completely negative results on *A. nuda* and *A. strigosa*, and on this character appear to be distinct from the Missouri strain C 2. This result was obtained in three separate experiments.

Before leaving the question of biological specialisation it is worthy of record that the *sativa* variety Markton, which remained immune in all trials conducted in the U.S.A., gave negative results in Wales with each of the seven biological species of *U. avenae* and *U. levis* which have been examined (Tables V and VI).

[1] It is not impossible that a collection like L 2, which produces infection on varieties belonging to widely different species of the host, may be a mixture of two or more biological species.

Table VI.

Showing the behaviour of four spore collections of U. levis *on seven varieties of oats.* 1926–27. *Reference C* 164.

Species and variety of host	Percentage infected plants produced by four spore-collections of *U. levis*			
	C 1, Wales	C 2, U.S.A.	C 3, England	C 4, England
Season 1926. Germinated in sand—				
A. strigosa orcadensis (521)	100	100	9	0
A. brevis (2384)	100	0	0	0
A. nuda (2495)	0	95	25	26
A. sativa, Potato (2855)	0	100	100	100
„ Victor (2394)	0	100	62	80
„ Grey Winter (2860)	0	95	96	96
„ Markton (2791)	0	0	0	0
Season 1927. Germinated in sand—				
A. strigosa orcadensis (521)	—	100	0	5
A. nuda (2495)	—	100	20	15
A. sativa, Grey Winter (2860)	—	100	100	90
Season 1927. Germinated on filter-paper—				
A. strigosa orcadensis (521)	—	100	—	0
A. nuda (2495)	—	100	—	28
A. sativa, Grey Winter (2860)	—	86	—	100

IV. The relative resistance of line selections of *Avena strigosa* to *U. levis*, biological species C 1.

In a previous paper reference was made to the varied behaviour of different lots of *A. strigosa* in regard to infection by covered smut (13). Data collected from published papers and from experiments carried out in Wales showed that certain lots were immune while others were markedly susceptible to the same race of smut (*loc. cit.* Table II).

During the seasons 1925–27, 101 line selections of *A. strigosa* were tested for resistance to the biological species of *U. levis* C 1. The data are summarised in Table VII. The most severe attack of smut was obtained in 1926 from grain sown in the open on March 31st. The heavy infection was probably due to similar conditions of rainfall and temperature which operated in experiment C 166 (Table IV). With shelled grain forty-eight lines suffered an attack of over 90 per cent. and in seventeen lines every plant of both rows was infected. The grouping of the lines in Table VII is based on these results.

Selections possessing extreme susceptibility occurred in each of the three sub-species of *Avena strigosa*. Of the resistant lines which gave under 10 per cent. infection with shelled grain in 1926, two belonged to

the sub-species *orcadensis*, twenty-seven to the sub-species *glabrescens*. Among the latter, nine remained immune in three seasons' tests.

It is evident that *A. strigosa*, in common with other species of *Avena*, includes varieties which vary within the widest possible limits in their reaction to smut fungi[1].

In any study of biological specialisation it is important that referenced pure lines should be employed as hosts for the parasites under investigation.

Table VII.

Showing a summary of infection experiments with pure lines of Avena strigosa *and* Ustilago levis, *biological species C* 1. 1925–27.

Sub-species and variety of *A. strigosa*	Ref. to group	Degree of infection 1926, shelled grain	No. of samples 1925	Grain in husk smutted tillers %	No. of samples 1926	Grain in husk smutted tillers %	Shelled grain smutted tillers %	No. of samples 1927	Shelled grain smutted plants %
Glabrescens—									
var. *cambrica*	I	Nil	9	0	10	0	0	10	0
„ „	II	Under 10 %	18	0·4	19	1·3	2·2	19	0·1
„ „	III	Over 10 %	20	6·3	18	41·6	75·2	5	39·2
var. *albida*	III	Over 10 %	1	13·0	1	46·0	93·0	1	24·1
Pilosa—									
var. *fusca*	III	Over 10 %	7	7·4	7	50·7	95·1	5	39·4
var. *alba*	III	Over 10 %	1	0	1	3·8	38·0	1	18·9
Orcadensis—									
var. *intermedia*	II	Under 10 %	2	0	2	1·7	6·4	2	0
„ „	III	Over 10 %	35	13·4	37	55·8	93·5	4	16·8
var. *flava*	III	Over 10 %	6	15·9	6	53·8	91·9	4	23·5

V. Observations on the natural smut infection of certain British oat varieties.

Data concerning the incidence of smut in commercial samples of oats grown at the Station during the seasons 1919 to 1922 were given in previous papers (16, 15). Among varieties most heavily infected are Potato and its allies, Ceirch du bach, Radnorshire Sprig and Tyrone Tawny.

Since 1923 a narrower range of varieties has been grown at the Station. Those named above continued to carry from time to time relatively severe attacks of loose smut, and in addition to these the following varieties yielded a considerable number of smutted panicles: *A. strigosa glabrescens* and *orcadensis*; *A. sativa*, Record, Grey Winter, Black Winter,

[1] The data are not sufficiently complete for publication, but evidence is available that selections of *A. strigosa* vary also in their resistance to loose smut (*U. avenae* L 11).

Black Bell and Lund. The species of smut on *sativa* varieties was invariably *U. avenae*.

A numerical estimation in 1926 of the attack of smut in a crop of Radnorshire sprig, described as "considerably smutted," showed that the infection amounted to $6 \pm 0{\cdot}1$ per cent. Records exist from other parts of the country of cases of smut attack which were estimated at 50 and 75 per cent. (7, 8), but these are probably of rare occurrence in the British Isles.

In 1927, 225 samples of oats were obtained from various districts of Britain and Ireland. They were sown in single (55 samples) or in duplicate (170 samples) rod rows at the farm on April 13th. Infected panicles were cut from each row as they made their appearance and the number taken was duly recorded for each sample. The species was in all cases *U. avenae*. Rows which produced more than one smutted panicle were ultimately harvested and the total number of fertile tillers was counted. The grain had been sown thickly and the panicles per row varied from 200 to 1000. The data are summarised in Table VIII. Nineteen samples were omitted as foreign, mixed, or not true to name.

The general intensity of the attack was slight. Only 5 samples produced more than 10 per cent. of smutted panicles. These belonged to either the Winter, Potato, or Sprig groups, the infection ranging from 11 to 33 per cent. (Table IX). Excluding 3 samples of Marvellous, all the samples (44) which yielded more than 1 per cent. of smut were representatives of the same three groups of varieties.

The trial included 55 samples of the newer *sativa* varieties of the group *verna* IV, and 70 samples of the sub-species *orientalis*. Considering these together 104 samples were entirely free from smut and 18 showed less than 1 per cent. infection. The data as a whole are in close agreement with those published by Stapledon (16).

The freedom from smut of the variety Black Tartar here and in previous trials is of interest in view of the fact that this variety showed marked susceptibility under experimental conditions (Table V). It is tentatively suggested that this and other varieties may be constitutionally susceptible, but liable to escape heavy infection by their method of flowering. Preliminary observations made in 1926 showed that some varieties rarely or never exserted their stigmas, while others, growing under the same conditions, opened their pales widely and exposed the stigmas fully. This habit would facilitate the contamination of the flower by spores of loose smut. The 5 samples which gave over 10 per cent. infection, 2 which were only slightly attacked and 1 which remained free from smut, served as material for a microscopic examination designed

Table VIII.

Showing the incidence of smutted panicles in 206 *samples of oat varieties from the British Isles.* 1927. *Reference C* 183.

Reference to group of varieties*	Number of samples in which smutted panicles occurred to the extent of					Total no. of samples
	0 %	Under 1 %	1–5 %	5–10 %	Over 10 %	
Avenae sativa—						
Sub-species *autumnalis*	9	1	4	4	2	20
„ *verna* (Group I)	1	2	5	0	2	10
„ *verna* (Group II)	9	13	12	1	1	36
„ *verna* (Group IV)	55	8	0	0	0	63
„ *verna* (Group V)	2	2	0	0	0	4
„ *orientalis*	57	10	3	0	0	70
Avena strigosa	2	1	0	0	0	3

* The groups included the following varieties. The figures in brackets indicate the number of samples of each variety.

A. sativa autumnalis. Bountiful (7), Black Winter (10), Grey Winter (3).

A. sativa verna. Group I. Radnorshire sprig (5), Sparable (1), Tyrone Tawny (1), Ceirch du bach (3).

Group II. Potato (16), Sandy (6), Tam Finlay (3), Long Houghton (1), Challenge (1), White Cluster (1), Castleton (6), Blainslie (2).

Group IV. Abundance (21), Victory (23), New Market (2), Svalöf King (1), Record (2), Crown (4), Fortune (2), Ascott (1), Besseler (1), Ligowo (1), King (3), White Horse (1), Banner (1).

Group V. Poland (1), Yellow Giant (1), Golden Rain (2).

A. sativa orientalis. Black Tartar (20), White Tartar (1), Superb (4), Supreme (10), Yielder (6), Harvester (2), Cropwell (1), Earl Haig (1), Marvellous (25).

to discover the resting form of the fungus on the grain. Twenty grains from each of 8 samples were examined by the method referred to above (p. 73). The results are shown in Table IX.

Chlamydospores, solitary or in clusters, were seen occasionally, but they were not so frequent or so abundant as a certain slender, branched and frequently budding mycelium which sometimes covered the inner surface of one or both pales (Plate IX, fig. 2 (*a*) and (*b*)). A coarser type of mycelium, often dark in appearance, occurred not infrequently, but it was usually easy to distinguish it from the more slender type, which stained particularly well with cotton blue. A comparison was made with that observed on the pales of grain which had been contaminated artificially with spores during the flowering stage. There seems little doubt that the slender type represents the resting form of *U. avenae* such as Zade (19) described. This identification is supported by the results quoted

in Table IX, since the number of grains carrying the mycelium was highest in samples which yielded the greatest number of smutted panicles in the field.

These data serve to emphasise the fact that the incidence of smut in any particular crop of oats is determined not only by the degree of infection in the parent crop but also by the climatic conditions during the period of flowering, since these will influence both the number and the germination of spores which are carried between the pales.

Table IX.

Showing the results of a microscopic examination of 20 grains taken from each of 8 samples included in experiment C 183 (Table VIII). Five of the samples produced over 10 per cent. of smutted panicles under field conditions in 1927.

Variety of *A. sativa*	Station number	District from which sample was obtained	Smutted panicles %	Grains showing gemmae and mycelium on the pales %
Sandy	3174	Durham	33	50
Bountiful	3319	Somerset	19	40
Ceirch du bach	3247	Pembrokeshire	15	35
Bountiful	3306	Devon	13	15
Radnorshire sprig	3298	Hereford	11	58
Sandy	3224	Wigtonshire	4	10
,,	3137	Belfast	2	0*
,,	3255	Montrose	0	0

* Chlamydospores were present on the inner pale of one grain, but none had germinated.

VI. Summary.

1. Various methods of testing varieties of oats for resistance to smut are described. A technique which involves the germination of shelled grain in sand of low moisture content at a temperature of 22° C. gave the most satisfactory results.

2. Data are given of the infection capacities of four biological species of *U. avenae* and three of *U. levis*. Two of these are described for the first time.

3. Pure line selections of *A. strigosa* vary in their resistance to *U. levis* (C 1) from 0 to 100 per cent.

4. A large number of commercial samples of oats was examined for smut infection. The attack was heaviest on varieties belonging to the Winter, Potato or Sprig groups. Samples yielding over 10 per cent. of smut were examined microscopically and typical resting mycelium of *Ustilago avenae* was found abundantly on the pales.

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The author is greatly indebted to Mr M. G. Jones, M.Sc., for samples of his own pure line selections of *Avenae strigosa*, and to Mr E. T. Jones, B.Sc., for most valuable help in connection with the classification of oat varieties. Special mention must be made of the detailed care given by Mr Watkins to the cultures described in the above paper.

Sincere thanks are given to Professor R. G. Stapledon for making available the facilities of the Welsh Plant Breeding Station, without which it would have been impossible to carry out the work, and for his constant interest and help.

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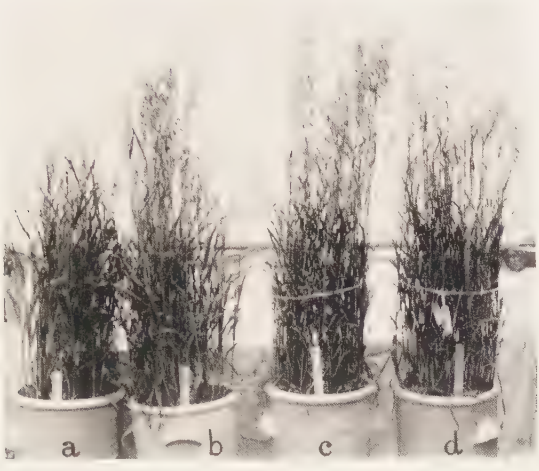

Fig. 1.

Fig. 2.

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EXPLANATION OF PLATE IX

Fig. 1. Showing the behaviour of four biological species of smut on *Avena strigosa orcadensis* (Cc 2662). Infection obtained by method (C). 1925.

(*a*) *U. levis* (C 1) ex Wales. 100 per cent. infection.
(*b*) *U. levis* (C 2) ex U.S.A. 80 per cent. infection.
(*c*) *U. avenae* (L 1) ex Wales. 0 per cent. infection.
(*d*) *U. avenae* (L 2) ex U.S.A. 50 per cent. infection.

Fig. 2. Oat pales carrying chlamydospores and mycelia identified as *Ustilago avenae*.

(*a*) Group of chlamydospores on the inner pale of the variety Sandy (Cc 3174 in Table IX). Natural contamination.

(*b*) Budding mycelium from another part of the same pale.

(*c*) Chlamydospores germinating on the surface of the inner pale of Potato oat (Table III, series C). Artificial contamination of exserted stigmas.

The drawings were made by the aid of a camera lucida under a 4 mm. objective and a 10× eyepiece. ×490.

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THE CONTROL OF "BUNT" IN WHEAT

By W. A. R. DILLON WESTON, M.A.
(*School of Agriculture, Cambridge.*)

The purpose of the following paper is to discuss the efficacy of the methods of treatment employed in the control of bunt in wheat.

Results of control measures against "Bunt" in wheat carried out on experimental plots at the Cambridge University Farm.

1. *A comparison between bluestone, formaldehyde and copper carbonate.*

It is the custom to arrange each year a series of demonstration plots showing methods of controlling this disease. These trials have included a comparison of the formaldehyde and bluestone methods of treatment; and, in the past two seasons, a comparison between the above methods and the so-called dust or powder treatment which consists in treating the grain in a dry way with a powder such as copper carbonate. The percentage of bunt is estimated at harvest by taking a count of 1000 heads in a diagonal band. The size of the plots is not standardised, but in any one year the plots are uniform in size. In 1924 each plot consisted of approximately 300 ft. of drill, in 1925 of 200 ft. and in 1926 of 100 ft.

The land generally available for these experiments is a poor light soil, and normally not one which would be seeded for wheat.

The wheat used is "Little Joss," and in every case prior to sowing this is very heavily artificially contaminated with bunt spores. When this wheat is sown in these experimental plots it is literally black, and no farmer normally would sow such wheat.

It is sometimes considered that this variety is more susceptible to bunt than other varieties, and, in order to determine if this is correct, twelve wheats, as follows, were contaminated at the rate of one part of bunt spores to twenty-five parts of wheat and sown under conditions as uniform as possible in field experiments. At harvest, Wilhelmina showed 73 per cent. of bunted ears, Rector 55 per cent., Benefactor 84 per cent., Squareheads-Master 68 per cent., Victor 71 per cent., Marshal Foch 67 per cent., Iron 69 per cent., April Bearded 89 per cent., Yeoman 82 per cent., Little Joss 72 per cent., Rivett 78 per cent., Red Marvel 80 per cent.

These figures suggest that Little Joss is not more susceptible than other English varieties.

Table I.

Result of four years' trials of bunt control.

Treatment and method	Year	Germination	% of bunted ears
Untreated	1921–22	100	55
	1923–24	98	63
	1924–25	94	87
	1925–26	99	90
Formaldehyde (1 : 240) 1 pint to 30 gallons. Treated by steeping method	1921–22	100	0·6
	1923–24	98	0·2
	1924–25	81	0·3
	1925–26	97	0·0
Copper sulphate 2½%. Treated by steeping method	1921–22	93	7
	1923–24	91	3
	1924–25	78	6
	1925–26	90	2
Copper carbonate 3 oz. per bushel. Treated by the dusting method	1924–25	97	15
	1925–26	99	10

The following figures show that the best method with heavily contaminated seed is the formaldehyde treatment. In interpreting these figures, however, it must be remembered that the wheat used had previously been artificially contaminated at an extremely high rate, as witness the percentage of bunt in the untreated plots. Such grain would not be used by the farmer for his seed wheat.

It is of interest to note that germination has only once been materially affected by the formaldehyde treatment, whereas in every case the use of copper sulphate has caused an appreciable reduction. The low germination with the formaldehyde and copper sulphate in 1924–25 was due very largely to the sample being one which was badly threshed, many grains having been broken. It will be seen that the dusting method, using copper carbonate, has not affected the germination. It must be noticed, however, that an infection of 87 and 90 has only been reduced by this treatment to 14 and 10 respectively, whereas the formaldehyde and copper sulphate treatments have reduced it considerably more than this.

2. *Concentration at which to use formaldehyde.*

At what concentration is it most economical to use formaldehyde?

Salmon has shown previously[1] that formaldehyde diluted 1 : 320 (1 pint to 40 gallons of water) was as effective in controlling bunt as the 1 : 240 solution (1 pint to 30 gallons of water). At a later date[2] Salmon used

[1] *Journal of the Ministry of Agriculture*, xxvii, 1921, 1013.

[2] *Journal of the Ministry of Agriculture*, xxix, No. 8, Nov. 1922.

formaldehyde solutions at the following dilutions: 1 pint of formaldehyde to respectively 40, 60, 80 and 100 gallons of water. The results obtained showed that the formaldehyde became less efficacious the more it was diluted below the 1 : 480 (1 pint to 60 gallons) limit, but that at this concentration it gave a perfectly satisfactory control of bunt.

In the season 1923–24 formaldehyde was used at three concentrations: 1 : 240, 1 : 480 and 1 : 640. Both the steeping and sprinkling methods were employed. Table II shows the results of control measures carried out against bunt in wheat for the season 1923–24 when 40 per cent. formaldehyde was used at different concentrations. From these figures it is clear that there is a wide range over which formaldehyde is both safe and effective. The strength now recommended in the Ministry of Agriculture's leaflet No. 92 is 1 pint of 40 per cent. formaldehyde to 40 gallons of water (*i.e.* a concentration of 1 : 320). This gives a safe and economical method of controlling the disease.

Table II.

Results of bunt control trials with formaldehyde of different strengths.

Material	Treatment: Strength	Treatment: Method of application	Result: Germination	Result: % of bunted ears
40 Formaldehyde	1 : 240 (1 pint to 30 gallons)	Sprinkled*	98	0·6
40 „	1 : 240 (1 pint to 30 gallons)	Steeped	99	0·4
40 „	1 : 240 (1 pint to 30 gallons)	Sprinkled*	98	0·2
40 „	1 : 240 (1 pint to 30 gallons)	Steeped	97	0·0
40 „	1 : 480 (1 pint to 60 gallons)	Sprinkled*	99	0·2
40 „	1 : 480 (1 pint to 60 gallons)	Steeped	99	0·4
40 „	1 : 640 (1 pint to 80 gallons)	Sprinkled*	97	0·2
40 „	1 : 640 (1 pint to 80 gallons)	Steeped	99	0·5
Untreated	—	—	98	63

* Sprinkled at rate of 1 gallon to 2 bushels.

3. *Dusting method.*

This method was first applied by Darnell-Smith in Australia. In the United States of America it is stated that copper sulphate represses root growth, and formaldehyde the development of the young shoot. It was on this account that Darnell-Smith's method was adopted in that country.

In order to test the value of dusts such as copper carbonate in controlling the disease, trials have been carried out during the past two seasons[1]. Various so-called "copper dusts" have been used which included copper carbonate, copper acetate, copper sulphate, copper stearate and copper arsenite.

[1] *I.c.* 1924–5, 1925–6.

In the season 1925–26 the copper carbonate used was drawn from a 2-ton consignment to Australia, shipped mainly for the purpose of seed wheat dressing.

Little Joss wheat was treated with varying amounts of crushed "bunt balls," sown, and the percentage of bunted ears estimated at harvest by taking a count of 1000 ears from a diagonal band across each plot, these consisting of six rows, each 16 ft. long.

Table III shows the percentage of bunted ears from untreated and treated samples, previously contaminated at different rates with crushed "bunt balls." It is clear from these figures that infection varies directly with the spore load and that the efficacy of the dusting treatment is proportional to the rate of contamination.

In the same season three plots were laid down in which contaminated wheat was dusted with varying amounts of copper carbonate (*i.e.* 3, 6, 9 oz. to the bushel). These results, showing the effect of an increased dressing of copper carbonate, are analysed in Table IV.

Table III.

The percentage of bunt in treated and untreated wheat contaminated at different rates.

Rate of treatment with bunt	Treatment	% of bunted ears
1 to 25	Copper carbonate 3 oz. per bushel	11
1 to 25	Untreated	94
1 to 50	Copper carbonate 3 oz. per bushel	5
1 to 50	Untreated	78
1 to 100	Copper carbonate 3 oz. per bushel	3
1 to 100	Untreated	50
1 to 500	Copper carbonate 3 oz. per bushel	1
1 to 500	Untreated	25
1 to 25	Untreated	92

Table IV.

Showing the effect that an increased dressing of copper carbonate has in reducing the percentage of bunted ears.

Treatment	Rate	Germination	% of bunted ears
Copper carbonate	3 oz. per bushel	99	24
,, ,,	6 oz. per bushel	99	14
,, ,,	9 oz. per bushel	99	6
Untreated	—	99	86

Practical aspect of dusting wheat. For the application of this treatment a dusting machine is required. There are several of these on the American, Australian and German markets. Of these the "Calkins" wheat-treating machine was used by the writer during the season 1926–27, and was found to work efficiently. The makers state that 30 bushels per hour can be treated with this machine, but, working experimentally, we were not able to reach this standard.

If no such dusting machine is available a suitable contrivance can easily be arranged from an old barrel or churn, or more simply by rolling a barrel containing the grain and powder. There is, however, one serious objection to this treatment, and that is the danger of inhaling the fine copper powder. This danger may be overcome by the labourer wearing a mask, or it can be minimised by carrying out the dusting operation in the open air. Using small hand machines, or home-made contrivances, the danger to the workman by this method may be considerable. Given, however, a good machine—that is, one which is dust proof—the operation should involve no danger—or even necessitate the wearing of a mask. Although it is not suggested that at the present this method should supplant the use of formaldehyde or bluestone, it is considered that this treatment is efficacious when dealing with large bulks of slightly contaminated wheat.

Relative costs for the three treatments with formalin, copper sulphate and copper carbonate.

Costs based on treatment of seed for two days' sowing: viz. ten sacks —acreage sown, sixteen acres.

Copper sulphate treatment by the steeping method.

	s.	*d.*
2 man hours	1	4
2 boy hours	0	8
Material ($2\frac{1}{2}$ per cent. sol. copper sulphate) (10 lbs. copper sulphate at 4*d.* per lb. to 40 gallons of water)	3	4
	5	4

Approximate cost per acre, 4*d.*

Formalin treatment by the steeping method.

	s.	d.
2 man hours	1	4
2 boy hours	0	8
Material (1 pint 40 per cent. formalin at 1*s.* 9*d.* per pint to 40 gallons of water)	1	9
	3	9

Approximate cost per acre, 3*d.*

Copper carbonate treatment by the dusting method, using a machine[1].

	s.	d.
4 men hours at 8*d.*	2	8
2 engine hours	2	0
2 oz. copper carbonate per bush. (5 lb. at 1*s.* 4*d.* per lb.)	6	8
	11	4

Approximate cost per acre, 8*d.*

With reference to the price of copper carbonate a firm of Commercial Chemists, ——, states:

When we sell in more or less large quantities for export to Australia, we charge 8½*d.* per lb. for 5-ton lots in casks and 9*d.* per lb. if in kegs; for a ton or less we charge 9½*d.* per lb., and quite small quantities anything from 10*d.* to 1*s.* per lb. It also depends upon whether we sell to merchants or to actual users, as of course merchants have to make something out of the business themselves. If your enquiry refers to a price that farmers in this country should pay, we suggest about 1*s.* 3*d.* per lb. for anything below 1 cwt., and about 1*s.* per lb. for 1 cwt. and over.

Although the cost of treatment by the dusting method is at least twice that of the others, it must be borne in mind that the great advantage of the dusting treatment is in its convenience, enabling as it does seedsmen to send out stocks already treated. It is difficult to convert this very appreciable advantage into terms of money.

Feeding treated wheat to poultry. Although it is inadvisable to feed poultry with dressed wheat, it is desirable to ascertain whether injury to poultry will result from feeding seed wheat so dressed. Feeding trials on dressed wheat were accordingly carried out by the Poultry Nutrition Section of the Animal Nutrition Institute, Cambridge, 4 oz. of dressed wheat being fed daily per bird to four White Leghorn hens over a period

[1] Depreciation on machine not taken into account.

of 5 days. The trials showed that, for short periods at least, such dressed wheat can be safely fed to birds. The samples of wheat fed had been dressed as follows:

Sample 1. Wheat steeped for 2 minutes in $2\frac{1}{2}$ per cent. copper sulphate solution.

Sample 2. Wheat steeped for 2 minutes in formaldehyde, strength 1 : 320.

Sample 3. Wheat treated with copper carbonate powder 3 oz. per bushel.

Analyses by Mr H. G. Pike showed that the dressed wheat contained 0·016 per cent. $CuSO_4.5H_2O$ and 0·101 per cent. copper carbonate respectively.

In concluding, the writer wishes to express his thanks to the following who have assisted him: Mr F. R. Petherbridge, with whom the writer co-operated in the 1923–24 experiments, Mr A. Amos for permission to reproduce the results of experiments in 1921–22, and for the figures relating to the costs of the three treatments, Mr E. T. Halnan for his report on feeding of "dressed" wheat to poultry, and Dr G. H. Pethybridge and Dr H. Hunter for constructive criticisms.

Summary.

1. Results of control experiments over a period of four years are given. The wet treatment, with formaldehyde and copper sulphate, are compared with the dry treatment, using copper carbonate.

2. The dusting method is discussed. It is shown that infection varies directly with the spore load and that the efficiency of the dusting treatment is proportional to the rate of contamination. For the usually slightly contaminated seed samples this treatment has been effective in controlling bunt.

3. The practical aspect of dusting wheat is discussed and its cost compared with the other treatments.

4. A report is given of the feeding of dressed wheat to poultry.

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THE ACTION OF SULPHUR AS A FUNGICIDE AND AS AN ACARICIDE

PART II

By WM. GOODWIN and H. MARTIN.
(*Research Department, South-Eastern Agricultural College, Wye, Kent.*)

(With one Text-figure.)

From the examination by chemical methods of the mechanism of the action of sulphur "at a distance" which formed the subject of our previous communication (3) certain conclusions were drawn which it was proposed to test by biological means. The main inference was that the agent concerned in this action, and which is formed when sulphur is applied to a heated surface, is elementary sulphur generated by volatilisation. This conclusion was based upon the fact that the agent, although reduced in amount by passage through a glass-wool filter maintained at ordinary temperatures was unaffected by filtration through a glass-wool plug heated to the temperature of the original sulphured surface.

The biological indicators employed were firstly, the powdery mildew of the hop (*Sphaerotheca humuli* DC., Burr.), chosen because it had been stated by Salmon (5) that under ordinary conditions actual contact of the sulphur particles with the fungus appears to be necessary for the fungicidal action to take place. Young leaves bearing vigorously growing patches of the "powdery" conidial stage of the fungus were selected for treatment, the other leaf at the same node being employed for the control experiment[1]. Secondly, the mildew *Erysiphe graminis* DC., growing upon couch (*Agropyron repens* Beauv.) was used, the couch being transplanted into pots prior to use. This fungus was chosen as the nearest approach available to the form growing upon young wheat plants, which was the one employed by Barker, Gimingham and Wiltshire (1). Only vigorously growing leaves bearing several densely powdery patches were selected for treatment, the control being a leaf as far as possible similar in growth. Thirdly, the black currant gall mite (*Eriophyes ribis* (Westw.) Nal.) was included as it was the organism concerned in Lee's experiments (4), and evidently was extremely susceptible to the action of sulphur

[1] The plants used were selected by Professor Salmon, who also kindly examined the fungus after treatment.

at a distance. It was found that twigs bearing big buds could be cut from the parent bush and kept in water without interference to the mites to an extent that would seriously affect the experiments.

It is not proposed to give in detail preliminary experiments designed for the determination of the optimum conditions of experiment, but to enter at once upon the discussion of the trials in their ultimate form noting, where necessary, the points observed in the preliminary work.

(*a*) Action as a fungicide.

The apparatus finally evolved is represented in Fig. 1. For the majority of the experiments recorded below the long tube contained a plug of glass-wool dusted with sulphur at (*a*). Below were two further plugs of clean glass-wool (*b*) and (*c*). The tube was thus divided into three portions, each of which was jacketed and the air (drawn from outside the laboratory) was blown first through the sulphured plug and then into the lower tube (*d*). The leaf, still attached to the parent plant, was placed within the tube (*d*) and was held in position by a copper clip. To protect the leaf tissue, a paper band (*e*) was gummed round the inside of the lower edge of this tube. For the control the apparatus was identical in every respect, except for the absence of sulphur or for a different treatment of the middle jacket; the leaves and also the patches of fungus were chosen as nearly as possible similar to those used in the experiment. The passage of the air through each apparatus was maintained at equal rates, whilst by a careful regulation of the heating it was possible to equalise the temperatures within the lower tube (*d*) just above the leaf surface. Strict control of this temperature, determined by a thermometer inserted at (*f*) was essential, for it was found that the use of temperatures much above 30° C. caused a collapse of the conidiophores, a condition which could not be prevented by increasing the humidity of the air. The air was in all cases initially saturated with moisture by passage through washing bulbs which also permitted the adjustment of the air speed.

From preliminary work it was found that even prolonged exposure had little if any immediate effect upon the powdery mildew of the hop. Thus in a series of experiments in which a composite glass-wool plug consisting of a wad of sulphur-dusted glass-wool supported by a wad of clean glass-wool to prevent the passage of actual sulphur particles removed by the current of air, was placed at (*c*), the control tube containing a glass-wool plug without sulphur, and the jackets heated by steam yielded the following results:

Sphaerotheca Humuli.

Exp. 27/13. Exposed 3 hours at average temperature in sulphur tube 24·9° C. (max. 25·5° C.), control tube 25·5° C. (max. 26·5° C.).

Examination with a hand lens showed that although on the second day after treatment some slight difference may have existed between the fungus patches of the treated and control leaves, by the fourth day the fungus was growing well on both leaves.

Exp. 27/14. Exposed 4 hours, average temperature in sulphur tube 27·2° C. (max. 28° C.), in control tube 28·7° C. (max. 31° C.).

Lens examination failed to show any difference in the health of the fungus upon the treated and control leaves. That the sulphur volatilised in the sulphur tube was reaching the surface of the leaf was established by placing thereon a small piece of clean copper foil. The foil became slightly tarnished in 30 minutes and was completely blackened within 90 minutes of the start of the treatment.

Exp. 27/15. Exposed 9 hours, average temperature in sulphur tube 27·7° C. (max. 29·5° C.), in control tube 29·1° C. (max. 30° C.).

Examination with lens on the first and fourth days after treatment showed no difference between treated and control leaves, upon both of which the fungus was apparently unaffected.

Preliminary trials similar in character made upon *Erysiphe graminis* gave strong indications of an action:

Erysiphe graminis.

Exp. 27/20. Exposed 4 hours, average temperature in sulphur tube 27·5° C. (max. 28·5° C.), in control tube 27·2° C. (max. 28° C.).

The fungus on the treated leaf showed immediate signs of change and a lens examination after 24 hours showed that all except one conidiophore upon the treated area had collapsed. Within 3 days a patch on the treated area showed a fresh growth of conidiophores. The fungus on the control leaf was apparently unaffected.

Exp. 27/21. Exposed 4 hours, average temperature in sulphur tube 26·4° C. (max. 29° C.), in control tube 26·4° C. (max. 29° C.).

Lens examination after 24 hours showed that the conidiophores upon the treated area of the leaf in the sulphur tube had collapsed and were of an ochraceous brown colour, but the radiating mycelium was still white; after 5 days certain of the mildew patches were again growing vigorously and glistening with conidiophores. The fungus on the control leaf was apparently unaffected.

Such results were definite indications that *E. graminis* was more rapidly affected by the sulphur volatilised from the glass-wool than *S. Humuli*, and the experiments carried out with the apparatus (A) shown in Fig. 1 were aimed not only at testing the removal of the volatile agent by its condensation and removal by the cooled glass-wool plug but also at establishing the existence of this specific action of the two fungi towards sulphur. Accordingly, after each apparatus had been heated till conditions were uniform and steam was passing from the exit tubes of

the lower jackets, the hop leaf, across which was placed the couch leaf, was fixed in position. In the second apparatus (B) containing similar plugs the middle jacket was cooled with cold water. The lower steam jacket enabled the maintenance of equal temperatures in the two tubes.

The following were the results obtained:

Exp. 28/42. Time of exposure 6½ hours, average temperature in tube A (all jackets steam heated) 26·5° C. (max. 29·5° C.), in tube B (middle jacket cooled) 27·3° C. (max. 28° C.).

Lens examination, after 24 hours, showed no signs of fungicidal action nor of differences in the fungi upon the leaves in tubes A and B.

Exp. 28/43. Tubes A and B as in Exp. 28/42, but no couch leaves employed. Exposed for 6½ hours, average temperature in tube A 22·3° C. (max. 23·5° C.), in tube B 22·5° C. (max. 24° C.).

After 24 hours there was no apparent difference between the two leaves and the fungus was apparently unaffected.

As in these two experiments it was found that even after several days no signs of fungicidal action developed, the leaf was, in subsequent experiments, removed from the parent plant after treatment and examined under the microscope, a procedure not adopted in the above experiments, since it was thought that indications of fungicidal action might be slow in appearing.

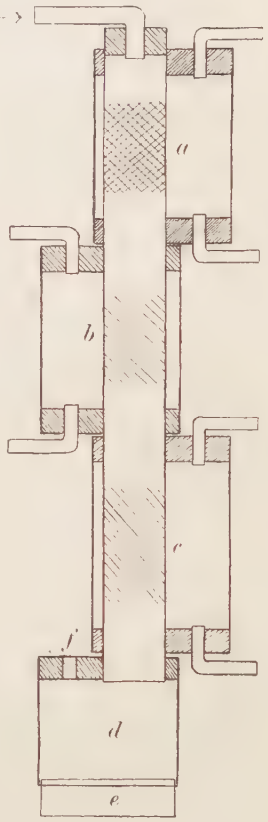

Fig. 1.

Exp. 28/44. As in Exp. 28/43, couch leaves placed over the hop leaves. Exposed for 5½ hours, average temperature in tube A 28·1° C. (max. 30° C.), in tube B 27·0° C. (max. 28·5° C.).

An examination with a lens immediately after withdrawal from the tubes showed no difference nor sign of action upon any of the treated leaves. After 24 hours, microscopic examination showed that with *S. Humuli* the fungus upon the leaf from tube A only showed a few shrivelled conidiophores—99 per cent. were healthy and upright; no signs of action were observed upon the leaf from tube B. With *E. graminis*, the leaf exposed in tube A showed many collapsed and shrivelled conidiophores, especially at the edges of the leaf, though certain areas bore healthy conidiophores. No signs of action were apparent upon the mildew patches from tube B. The copper clip used to retain the leaves in position was at the conclusion of the exposure markedly tarnished in tube A, that in tube B showing but the slight dullness to be expected after exposure to air.

Exp. 28/45. As in the above, time of exposure 7 hours, average temperature of tube A 28·5° C. (max. 30° C.), of tube B 27·8° C. (max. 29° C.).

Microscopic examination revealed that on both the hop and couch leaves treated under tube A the fungus was affected, the chains of conidia were shrivelled to the extent of 50 per cent. or over. Of the leaves treated under tube B the fungus upon the couch leaf showed no signs of fungicidal action, whereas in the case of *S. Humuli* although there were certain conidiophores showing spores either partly or wholly shrivelled, their number did not approach 50 per cent.

Exp. 28/46. As in the above, time of exposure 6 hours, average temperature in tube A 29·5° C. (max. 31·5° C.), in tube B 29·0° C. (max. 31° C.).

The copper clip under tube A had developed a reddish stain 2 hours after placing in position. Microscopic examination showed that on both the hop leaves the fungus was affected in certain areas. Upon the couch leaves, that under tube B was apparently unaffected; whilst upon the leaf from tube A one patch of mildew showed many shrivelled conidiophores, an adjacent patch was apparently healthy.

Exp. 28/47. As in the above, time of exposure 9½ hours, average temperature in tube A 28·7° C. (max. 31° C.), in tube B 28·7° C. (max. 31° C.).

Upon all the areas treated in both tubes A and B there were but slight signs of fungicidal action—occasional conidia were shrivelled. It was found, however, that even on an untreated leaf of the hop the fungus had a similar appearance.

These last six experiments, which were the concluding ones of a long series, show well the failure to secure conclusive results when employing fungi as the test material. This inconclusiveness is due primarily to the slowness of the action of sulphur upon the fungus, a point to which we must return later. A further important factor was the failure to secure controls completely healthy, a failure to be attributed mainly to two facts, firstly, the rapid fungicidal action of hot air, secondly, the shrivelling of the conidia under normal conditions. It was thought that possibly the action of the hot air might depend upon the desiccation of the conidia, but trials in which the treated leaf was placed upon filter paper kept continually moist still showed shrivelled conidia at the end of the exposure. As regards the normal shrivelling of the conidia the nature of the experiment prevented a preliminary microscopic examination of the fungus, and so the existence of shrivelled conidiophores prior to treatment could not be established except by reference to untreated patches of the mildew. Yet another difficulty was introduced into the microscopic examination of the fungus after treatment owing to the effects of the treatment being localised to a remarkable extent. In many cases it was found that although at one point the fungus would be completely shrivelled and collapsed, an adjacent area would show little sign of fungicidal action. It was therefore necessary to examine the mildew patches at many points before arriving at a final conclusion which was of necessity frequently

indefinite in character. This curious localised action it is suggested may be due to a failure of the air to penetrate the densely packed conidiophores, for it was often observed that signs of action were confined to the edges of the mildew patch. If such be the case it would be analogous to the condition found in the use of fungicidal sprays, where without the addition of a spreader the patches could not be uniformly wetted (2).

Although the experiments must be considered to have failed in their initial objects, (1) to show the removal by filtration through a cooled wad of glass-wool of the volatile agent formed when sulphur is heated, (2) to establish the specificity of the fungi *S. Humuli* and *E. graminis* towards the action of sulphur, they throw considerable light upon the mode of action of sulphur as a fungicide. It was evident that the amount of sulphur volatilised when heated to almost 100° C. for 8 to 9 hours was insufficient to cause a complete collapse of the conidiophores of the fungi. In view therefore of the enormous reduction of the vapour pressure of sulphur with temperature the vaporisation of the sulphur could play only a small part in the exertion of its fungicidal action at ordinary temperatures. Strong support is therefore given to the view that actual contact of the fungus with the sulphur particle is necessary before it is killed. In many cases the microscopic examination revealed apparently healthy and turgid conidia bearing many small particles of sulphur, an indication that in the action of sulphur at a distance the re-condensation of the volatilised sulphur upon the fungus is part of the mechanism of the fungicidal action and that sulphur vapour is not directly toxic to the fungus.

These opinions were therefore tested by experiments in which the leaf bearing many vigorously growing mildew patches was placed inside a glass tube coated on the interior with sulphur. The plant was placed in an unheated glasshouse and the temperature within the tube recorded by means of a thermometer.

Exp. 27/42. One hop leaf was enclosed in a sulphured tube, whilst the opposite leaf at the same node was dusted with sulphur and placed in a similar tube. The leaves were exposed from 8. vi. 27 to 12. vi. 27; the temperatures being read at 9 a.m., 12 noon, 3, 6 and 9 p.m. yielded an average of 21° C. (70° F.) with a maximum of 33° C. (91·5° F.). Although the patches of mildew dusted with sulphur showed, at the end of treatment, the characteristic fungicidal action of sulphur, those upon the leaf in the sulphured tube were apparently unaffected.

Exp. 27/43. Three tubes, two of which were arranged as in Exp. 27/42, a third containing a leaf upon which "colloidal" sulphur had been painted around a vigorously growing patch of *S. Humuli*, were set up. The leaves were exposed from 13. vi. 27 to 16. vi. 27; the temperatures read at the same intervals as in Exp. 27/42 yielded an

average of 24·7° C. (76·5° F.) with a high maximum of 40° C. (104° F.). Examination on the 19th, three days after the extremely hot day, showed that on the control leaf the tips of certain conidiophores were withered. Whilst the fungus dusted with sulphur was showing the typical signs of fungicidal action no difference could be observed between the control and the fungus on the leaf placed in the sulphured tube nor even between the control and the mildew patch surrounded by "colloidal" sulphur.

Exp. 28/53. Six tubes were arranged as in the above experiments, three containing couch leaves bearing vigorously growing patches of *E. graminis*, three containing hop leaves bearing patches of *S. Humuli*. One of each set served as control, whilst the other two tubes were painted on the inside with sulphur. The plants were placed in an unheated glasshouse from April 5th to May 21st (46 days). Upon removal, all the mildew patches were found to be growing vigorously despite the presence of a parasitic fungus which had itself developed in the presence of the sulphur.

These results definitely indicate that the production of volatile sulphur at ordinary temperatures is insufficient to bring about the toxic action known to be possessed by sulphur against these two fungi.

(*b*) Action as an acaricide.

Preliminary experiments indicated that the black currant gall mite is extremely sensitive to sulphur and that the organism is affected even by the small amount of sulphur volatilised at ordinary temperatures. The following are the results of a series of trials carried out with an apparatus similar to that employed for experiments 27/13–15 recorded in section (*a*). In the sulphur tube was placed a composite glass-wool plug consisting of glass-wool dusted with sulphur supported by a clean glass-wool wad, the control tube contained a clean glass-wool plug, whilst the outer jacket of both tubes was heated by steam. Twigs bearing big buds from which the mites were actively emerging were placed in the lower tubes, the bud being adjacent to the bulb of the thermometer.

Exp. 28/81. Buds exposed 1 hour, temperature in both tubes 17·5° C.

Examination under a binocular microscope showed that, whereas the mites upon the control bud were moving vigorously, the majority of those on the bud exposed in the sulphur tube were motionless and apparently dead.

Exp. 28/82. Buds exposed 40 minutes, temperature in both tubes 21·5° C.

Examination with the microscope 30 minutes after treatment showed no movement on the bud exposed in the sulphur tube, approximately 10 per cent. of the mites on the control bud were moving, a percentage which was markedly increased when examined an hour later.

It was evident that if movement was to be accepted as the criterion of non-acaricidal action, the buds after treatment should be exposed to conditions which would be favourable to the movement of any mites still alive. It was found that at low temperatures the mites would become

extremely sluggish and movement could in most cases only be detected in the slow waving of the legs. Such mites on exposure to sunlight or when placed near the steam oven again quickly became active. In the majority of the experiments recorded below counts of the moving mites were made after exposure for at least 90 minutes to sunlight or warmth. Owing to this delay in taking the counts it was found that a certain number of active mites were observed even on the treated buds, active probably because they had emerged from the bud subsequent to the treatment. As was to be expected, it was found that exposure of the bud to sulphur vapour did not affect the mites still hidden inside the bud and so protected.

Further, it was usually found that a small number of mites upon the control buds were motionless and in many cases obviously dead. Their death may have been due to desiccation or to natural mortality, for they were frequently observed even upon untreated buds.

The counts were carried out upon at least five different fields obtained by placing the bud in various positions under the microscope, the average percentage of mites moving being recorded below.

Exp. 28/85. Buds exposed for 6 hours, both tubes unheated and temperature at buds 13·5° C.

Examined after exposure to sunlight, 24 hours after the start of the treatment:

	Total no. mites examined	% mites moving
Control tube	95	85·3
Sulphur tube	61	6·6

Exp. 28/95. Buds exposed 3½ hours, both tubes unheated and temperature at buds 18·0° C.

Examined next day after exposure to sunlight:

	Total no. mites examined	% mites moving
Control tube	367	67·8
Sulphur tube	204	34·3

Exp. 28/96. Buds exposed 7 hours, tubes unheated and at average temperature 22·0° C.

When examined immediately after treatment it was found that the mites upon the control bud were apparently unaffected, the bud being densely packed with moving mites too numerous to count. Upon the bud from the sulphur tube, of the enormous number of mites observed, only three could be found showing signs of movement.

It is interesting to note that in the chemical part of this investigation it was found that clean copper foil was but slowly tarnished by exposure to air passed over sulphur heated at 38° to 40° C., the black currant gall

mite would appear to be a more sensitive reagent for the detection of sulphur vapour.

In view of this sensitivity of the mite towards sulphur vapour it was apparent that in using the three-jacketed apparatus (Fig. 1) a high mortality was to be expected, even in the control apparatus, where the greater part of the sulphur vaporised in the upper part of the tube was removed by the cooled glass-wool plug. The experiments, however, all showed a marked reduction in the percentage of mites affected by the passage of the air through the cooled filter:

Exp. 28/90. Buds exposed 3 hours, counts made after exposure for 1½ hours to sunlight:

	Tube A (all jackets heated)	Tube B (middle jacket cooled)
Average temp. of bud ° C.	15·1	15·0
Total no. mites observed	201	178
% mites moving	2·5	20·2

Exp. 28/92. Buds exposed 1½ hours, counts made 24 hours after treatment and after exposure for 1½ hours to sunlight:

	Tube A	Tube B
Average temp. of bud ° C.	17·1	16·5
Total no. mites observed	307	273
% mites moving	3·0	41·0

Exp. 28/93. Buds exposed 1½ hours, counts made 20 hours after treatment and after exposure for 1½ hours to sunlight:

	Tube A	Tube B
Average temp. of bud ° C.	18·7	18·8
Total no. mites observed	142	165
% mites moving	5·0	35·7

As a check, these buds were afterwards placed for 1 hour near the steam oven; recounts yielded the following results:

Total no. mites observed	334	208
% mites moving	6·9	43·3

Exp. 28/94. Buds exposed 1 hour. A control trial in which the bud was left untreated was included in this experiment which gave the following results:

	Tube A	Tube B	Control
Average temp. of bud ° C.	19·5	19·5	16·0
Total no. mites observed	187	194	201
% mites moving	12·8	36·6	83·6

From these experiments it is permissible to conclude that the agent responsible for the death of the gall mite and which is evolved when sulphur is heated is gaseous sulphur. Its amount is diminished by passage through a cooled glass-wool filter, though evidently this condensation

and filtration process still permits the passage of sufficient volatile sulphur to produce a marked effect upon the gall mite.

As it has been suggested that sulphur dioxide or hydrogen sulphide are the agents formed from heated sulphur and responsible for its action at a distance, experiments were carried out to determine the action of these gases upon the gall mite. Similar trials proved unsatisfactory in the case of the fungi owing to the direct action of these gases upon the leaf and the possibility that their action upon the fungus was therefore indirect.

For the purpose of these experiments the air was first passed, at a rate of approximately 500 c.c. per minute, through a dilute solution of sulphuric acid to which could be added by means of a drop funnel, in the one case, measured amounts of a 10 per cent. solution of crystalline sodium sulphite, $Na_2SO_3.7H_2O$, in the other case, measured amounts of a solution of crystalline sodium sulphide, $Na_2S.9H_2O$. These solutions were freshly prepared for each experiment.

Trials in which the bud was held for 1 minute and for 10 minutes in an atmosphere smelling strongly of sulphur dioxide and in which moist blue litmus paper was immediately reddened showed that the mites, although perhaps at first affected, rapidly recovered on exposure to sunlight. In one experiment the bud was exposed for $4\frac{1}{2}$ hours during which time 0·051 gm. sulphur dioxide passed through the apparatus (average concentration approx. 0·013 per cent. by volume sulphur dioxide). Examination of the bud after 24 hours showed no difference between the mites of the treated bud and those on a control bud exposed to a similar stream of air drawn from outside the laboratory. Even in a trial in which the bud was exposed, at 18° C., for 5 hours to an average concentration of 0·029 per cent. by volume sulphur dioxide, no apparent effect upon the mites was observed though a small leaf adjacent to the bud was wilted after the treatment.

Similarly, experiment showed that hydrogen sulphide at such concentrations was without permanent effect upon the mite. After an exposure, at 20° C., for 90 minutes to a concentration of 0·208 per cent. by volume hydrogen sulphide followed by exposure to sunlight for 1 hour, the mites were found to be less active upon the treated bud. No difference, however, could be detected between the mites and those of a control bud after exposure to sunlight for 3 hours.

Summary.

The conclusion arrived at by chemical methods, that the volatile agent produced when sulphur is applied to a heated surface is gaseous sulphur, has been subjected to biological tests in which the fungi *Erysiphe graminis* and *Sphaerotheca Humuli* and the gall mite *Eriophyes ribis* were employed.

The fungi were found not to be sufficiently sensitive to yield satisfactory and concordant results and strong support is given to the view that actual contact of the sulphur particle with the fungus is necessary before fungicidal action can occur.

It was shown that the agent present in air passed over heated sulphur and responsible for the death of the gall mite was not removed by filtration through a heated glass-wool plug, this observation being contrary to the view that the toxic agent is produced initially in solid form.

Filtration through a cooled glass-wool plug only removed part of the volatile agent and it was shown that the gall mite is affected by the traces of sulphur volatilised at ordinary temperatures.

The results of the experiments with the gall mite were in complete accord with those obtained in the previous chemical work.

At relatively large concentrations sulphur dioxide and hydrogen sulphide are without permanent effect upon the gall mite and these gases are therefore not responsible for the acaricidal action of sulphur.

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ON THE OCCURRENCE OF THE PARTHENOGENETIC AND SEXUAL FORMS IN *APHIS RUMICIS* L., WITH SPECIAL REFERENCE TO THE INFLUENCE OF ENVIRONMENTAL FACTORS

By J. DAVIDSON, D.Sc.[1]

(*Rothamsted Experimental Station, Harpenden.*)

(With 6 Text-figures.)

CONTENTS.

I. INTRODUCTION.

In an earlier paper (5) the writer gave a short review of the more recent literature dealing with the occurrence of alate and apterous parthenogenetic females in aphides. Since that time several investigators have brought forward experimental evidence showing the influence of external factors on the occurrence of these forms, notably Brittain (3), Mason (17),

[1] Now at the Waite Agricultural Research Institute, University of Adelaide, South Australia.

Wadley (23), Ewing (11), Ackerman (1) and Reinhard (19). In most cases these writers have briefly summarised the views of previous observers so that it is not necessary to do so here.

The older views regarding the influence of environmental factors on the occurrence of the sexuales in aphides were also briefly discussed in my earlier paper (5). Little experimental work has been done on this problem, although it is apparent from field observations on several species that external factors play a large part in bringing about the cyclical change in these insects. Since Klodnitzki's (1912) extensive studies, which were dealt with in my previous paper, two further contributions to the literature are of particular interest. Uichanco (21) discusses the modifying influence of environmental factors on reproduction in the Aphididae and a later paper (22) contains an excellent account of the embryogeny and post-natal development of the Aphididae. Marcovitch (14, 15) shows the importance of length of day as a factor affecting migration and the occurrence of the sexual forms in aphides.

In the present paper an account is given of the results obtained with *Aphis rumicis* L. (*A. fabae* Scop.) from rearing experiments carried on during the past $7\frac{1}{2}$ years, particularly with reference to the two phenomena of the life-cycle referred to above.

The aims in view were (*a*) To trace the normal sequence of the generations in the complete life-cycle and the occurrence of the parthenogenetic and sexual phases. (*b*) To see whether the normal sequence could be affected experimentally by changing the environment in which the aphids were reared. (*c*) To observe the influence of environmental changes on the occurrence of alatae and apterae.

The experiments were commenced in June 1920 (Line A) with two apterous viviparous females taken from a wild colony on beans found in a local garden, and nine further related parthenogenetic lines were reared during succeeding years.

II. METHODS OF REARING THE VARIOUS PARTHENOGENETIC LINES.

The methods employed in rearing the aphids have been already described (9). The relationships of the ten parthenogenetic lines are shown in Fig. 1 and Table I. In each of the eight lines, started with a Fundatrix, the aphids were reared on *Euonymus europaeus* until alate migrants developed (usually in 3rd generation), and afterwards on Longpod beans until the line was discontinued. In each year, about the end of August, colonies from the beans were established on *Euonymus* so as to ensure that sexuales would be obtained and fertilised eggs laid. When the

Table I.

Showing relationships of the 10 *parthenogenetic lines of* A. rumicis.

Line	Started with	Taken from line	Parthenogenetic generations		Ova		
			Started	Ended	Laid		Commenced to hatch
A	2 apterae	Wild colony	16. vi. 20	30. v. 21	Oct.–Nov.	1920	8. iii. 21
B	Fundatrices	A	8. iii. 21	31. xii. 23	Oct.–Nov.	1921	23. iii. 22
					,,	1922	None hatched
					,,	1923	17. iii. 24
C	,,	B	23. iii. 22	28. ii. 23	Transfers not made for this purpose		
D	,,	B	23. iii. 22	8. ii. 23			
E	,,	B	17. iii. 24	30. xi. 24	Oct.–Nov.	1924	7. iv. 25
F	,,	E	7. iv. 25	21. vi. 26	,,	1925	24. iii. 26
G	,,	F	24. iii. 26	30. xi. 26	,,	1926	15. iii. 27
H	,,	G	15. iii. 27	31. xii. 27	,,	1927	—
Ha	Apterae	H	6. v. 27	31. xii. 27	,,	1927	20. iii. 28
I	Fundatrices	G	20. iii. 27	9. xi. 27	,,	1927	—

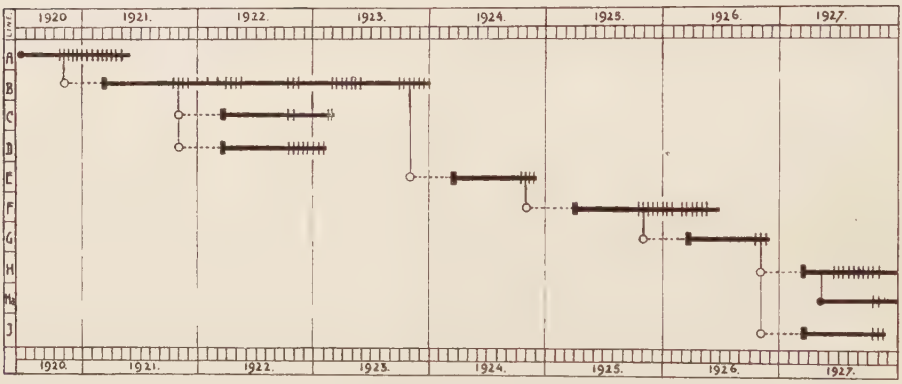


Fig. 1. Chart showing the relationships of the ten parthenogenetic lines of *Aphis rumicis* used in the experiments.

The years are divided into monthly periods. The broad black bands show the periods during which continuous parthenogenetic reproduction was maintained in each parthenogenetic line: the short cross-lines indicate the periods during which sexual forms occurred in the colonies: where the cross-lines are absent, only parthenogenetic individuals were obtained. Line B was started from one fertilised egg taken from line A and lines C–I are of the same strain, being related as shown with the thin connecting vertical lines.

With the exception of lines A and Ha each one was started from a fertilised egg.

○ =fertilised ovum. ▬ =fundatrix. ● =apterous viviparous female.

During the winter period the aphids were reared under varying temperatures and with line H they received only 8 hours daylight daily from March to November. See also Figs. 4 and 5.

fundatrices began to hatch out the following spring, new lines were started as shown in Fig. 1.

Line A was started from a wild colony. Line B was started with one fundatrix from line A and since the subsequent lines were descended from line B, a single strain of the species has been used throughout. The aphids were reared in a large open glasshouse during the summer period, or in the open air insectary and in a heated glasshouse during the winter period. The air temperature at Rothamsted during the $7\frac{1}{2}$ years is shown in Figs. 3 and 4 and also the temperature during the winter period in the heated glasshouse. The temperature in the summer glasshouse (1920–21) and open-air insectary (1927), during the summer period, is also shown. It will be seen that the temperature in the insectary approximates closely to that of the outside air temperature plotted from Rothamsted records and the temperature in the glasshouse during the summer period shows an average mean about 10° F. higher than the outside air temperature.

The *Euonymus* plants on which ova were laid in autumn, were kept outside in the open air during winter, being removed to the summer glasshouse in spring, soon after the fundatrices commenced to hatch out. By reference to Figs. 3 and 4 the conditions under which the aphids were reared can be seen from the following data: the symbols *O* = open air; *O.G.* = summer glasshouse; *H.G.* = heated winter glasshouse. It will be noted that the dates given for aphids in the open air (*O*) refer to living aphids; the ova from which the fundatrices were obtained were kept outside during winter.

Line A. 16. vi to 12. x. 20 (*O.G.*); 13. x. 20 to 31. iii. 21 (*H.G.*), 1. iv to 30. v. 21 (*O.G.*). *Line B.* 8. iii to 31. iii. 21 (*O*); 1. iv to 9. x. 21 (*O.G.*); 10. x. 21 to 20. iii. 22 (*H.G.*); 21. iii to 31. x. 22 (*O.G.*); 1. xi. 22 to 28. iii. 23 (*H.G.*); 29. iii to 19. x. 23 (*O.G.*); 20. x to 31. xii. 23 (*H.G.*). During the period 12. xi. 22 to 12. i. 23 the colonies received artificial light from electric lamps in addition to normal daylight (see Davidson, *Journ. Sci.* 1924, LIX, p. 364). A control series under the same temperatures received only normal daylight. *Line C.* 23. iii to 31. x. 22 (*O.G.*); 1. xi. 22 to 28. ii. 23 (*H.G.*): as in line B the colonies received artificial light, control colonies receiving only normal daylight. *Line D.* 23. iii to 31. x. 22 (*O.G.*); 1. xi. 22 to 8. ii. 23 (*H.G.*): the temperatures for this line, during the winter period, were lower than for B and C as can be seen in Fig. 3 (1927, middle line). *Line E.* 17. iii. to 30. xi. 24 (*O.G.*). *Line F.* 7. iv to 19. iv. 25 (*O*); 20. iv to 8. xi. 25 (*O.G.*); 9. xi. 25 to 20. iv. 26 (*H.G.*); 20. iv to 21. vi. 26 (*O.G.*). *Line G.* 24. iii to 30. xi. 26 (*O.G.*). *Line H.* 15. iii to 20. iv. 27 (*O.G.*); 21. iv to 30. ix. 27 (open-air insectary); 1. x to

31. xii. 27 (*H.G.*). From 28. iii to 5. ix. 27 the colonies in this line received only 8 hours daylight daily, being placed in a dark box from 5.30 p.m. until 9.30 a.m. daily. From 25. x to 23. xii. 27 the colonies were submitted to artificial light from electric lamps from sunset until 10 p.m. during 5 days each week: a control series reared under the same temperatures received only normal daylight, being kept in a dark box during the illumination period. *Line Ha.* 15. iii to 20. iv. 27 (*O.G.*); 21. iv to 30. ix. 27 (open-air insectary); 1. x to 31. xii. 27 (*H.G.*): this was a control line for line H, and after 5. v. 27 the aphids received normal daylight but, as in line H, a series of colonies received artificial light during the period stated and a control series had only normal daylight. *Line I.* 20. iii to 20. iv. 27 (*O.G.*); 21. iv to 9. xi. 27 (open-air insectary): this was a further control line for line H and the aphids received normal daylight.

III. NORMAL OCCURRENCE OF THE PARTHENOGENETIC AND SEXUAL FORMS.

The normal life-cycle of the bean aphis as it occurs in England is shown graphically in the following diagram, together with the terms used in the present paper. A reference to this diagram will enable the reader to follow more readily the details discussed later.

IV. NORMAL OCCURRENCE OF PARTHENOGENETIC ALATAE AND APTERAE.

It is clear from Fig. 2, that, if the normal bi-sexual cycle is to be completed, alatae must develop during two critical periods of the cycle, namely in spring when migration takes place from the winter host to the summer food-plants and again in autumn when the alate sexuparae (re-migrants) are due to return to the winter host plant. Furthermore, during the summer period, owing to the rapid reproduction of this species, it is necessary that alatae develop from time to time so as to prevent the starvation of the aphids in overcrowded colonies, and to ensure the distribution of the species to other food-plants. Since the sexual females (apterous) are produced by the alate sexuparae, it follows that, if experimental conditions are established such that a continuous line of apterous parthenogenetic females only are produced, the normal bi-sexual cycle cannot be completed. The occurrence of alatae therefore is closely associated with these three important features of the life-history and the environmental factors which influence migration and the change from the parthenogenetic to the sexual method of reproduction must also be considered in this respect as exercising an influence on the occurrence of

apterae and alatae. The alate form is the primitive condition and it seems to the writer, as already stated in a recent paper (10), that the logical interpretation of the problem of the occurrence of the alatae and apterous parthenogenetic females is, What factor or factors make for the occurrence of apterae? since by the continuation of a purely apterous parthenogenetic line, the distribution of the species is severely limited and the completion of the normal bi-sexual cycle is prevented.

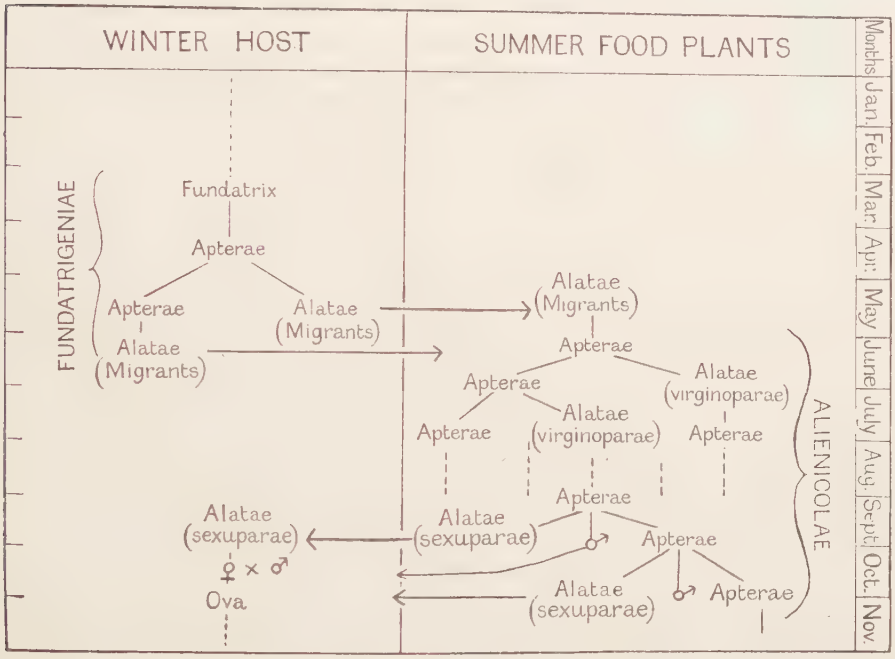


Fig. 2. Diagram illustrating the life-cycle of *Aphis rumicis*, showing the terms used for the different forms in the various generations.

V. THE FUNDATRIGENIAE GENERATIONS.

The fundatrigeniae are the descendants of the fundatrix, born on the winter host (Fig. 2) and consist of apterae and alatae, the latter being the migrants.

(*a*) *Offspring of the fundatrices.*

The offspring of the fundatrices are usually apterous but may consist of a mixed brood of apterae and alatae, the former being usually in the majority. The proportion of alatae which develop is affected by the condition of the food-plant (nutrition factor) and by the amount of young growth available in relation to the size of the colonies (overcrowding).

Counts made of the individuals present in four separate colonies on *Euonymus* produced by four fundatrices, after periods varying from 12 to 19 days, gave the following percentages of apterae—100, 83, 97, 11·5. The latter plant was "woody" and not making young growth, whereas on the other three plants there was plenty of young growth. Other instances were observed in which fundatrices produced a majority of alatae on "woody" *Euonymus* plants. When young shoots were maintained by cutting back the plants and overcrowding avoided, apterous fundatrigeniae produced a larger proportion of apterae in several successive generations. Fundatrices reared on Longpod beans and on *Rumex* produced freely and the offspring developed chiefly into apterae.

(*b*) *Offspring of the fundatrigeniae apterae.*

The offspring of the apterae of 2nd generation on *Euonymus* develop chiefly into alatae (3rd generation) and although a few apterae may occur in this 3rd generation their offspring develop into alatae, so that after about three generations the spindle tree becomes free from the aphis, owing to this tendency for alate migrants only to develop. This sequence under natural conditions is correlated with overcrowding and nutrition, and the condition of the spindle tree does not favour the development of apterae when the flush of early spring growth is over. There is, however, apparently an inherent tendency for alate migrants to develop in these early generations on *Euonymus*, which is evidently associated with the evolution of the migrating habit. This tendency is affected by overcrowding and nutrition. Consequently, the proportion of alatae and apterae in a colony may vary considerably according to these conditions.

Table II.

Showing offspring of fundatrigeniae apterae reared on Euonymus (1–7) *and beans* (8, 9).

Colony no.	Date apterae transferred	No. transferred	Alatae and apterae in colony after 10 days		
			Days	Apterae	Alatae
1	25. iv. 14	2	25	0	Many
2	5. iv. 21	5	21	Few	„
3	5. iv. 21	6	21	„	„
4	30. iv. 22	1	19	0	58
5	25. iv. 22	1	21	1	44
6	30. iv. 22	1	19	1	55
7	9. v. 27	9	19	20	400
8	6. v. 27	2	18	19	2
9	6. v. 27	6	18	71	27

If they are favourable, apterae may be obtained in several successive generations.

In Table II (Nos. 1–7) examples are given showing the strong tendency for the offspring of apterae to develop into alatae when reared on *Euonymus*.

When reared on beans a higher proportion of apterae developed as shown in Nos. 8 and 9. Similarly on *Rumex* the proportion of apterae was greater. This appears to be due to the better nutrition afforded by these plants.

(*c*) *Offspring of the fundatrigeniae alatae* (*migrantes*).

The offspring of the migrantes are normally laid on intermediate food-plants and develop into apterae. When reared on *Euonymus*, on which plant they reluctantly reproduce, apterae develop if suitable young growth is available, but if the plant is "woody," alatae will also develop, which is further evidence of the unsuitability of *Euonymus* as a permanent food-plant and of the effect of nutrition on the occurrence of alatae. When successive generations, from the fundatrix, are reared on *Euonymus*, alatae tend to predominate (*vide* (5), p. 305)[1].

VI. THE ALIENICOLAE GENERATIONS.

The alienicolae generations are initiated by the alate migrants and we have seen that the unsuitability of the spindle tree as a summer food-plant and overcrowding are important factors affecting the progress of the rhythmical spring migration. The descendants of the offspring of alate migrants can be considered as alienicolae so long as an unbroken line of parthenogenetic generations is maintained. In line B, for instance, a line of alienicolae was carried on for $2\frac{1}{2}$ years.

The following forms may develop in the alienicolae generations: (*a*) apterous viviparous females; (*b*) alate viviparous females (virginoparae) which produce parthenogenetic viviparous females; (*c*) alate viviparous females (sexuparae) which produce apterous sexual females; (*d*) alate males. The occurrence of these various forms is influenced by environmental factors, particularly length of day, overcrowding, nutrition and temperature. In nature, continuation of the parthenogenetic

[1] It is of interest to note in this respect that the autumn re-migrants (sexuparae) lay the sexual females below the old leaves of the spindle tree and the latter feed along the midrib and secondary veins, until they go to the branches in order to lay their eggs. The sexual females, therefore, are not dependent upon young growth. On the other hand, the spring migrants and also the alate virginoparae of the summer generations lay their offspring on or near the young growth of plants, on which the latter instinctively feed.

generations over the winter period is limited by seasonal and climatic factors. Under experimental conditions, however, favourable temperature and nutrition may be maintained and parthenogenetic reproduction carried on for long periods. Even under these conditions, the progress of the colonies during the winter period is slow compared with the summer period indicating the importance of the light factor[1].

A. Occurrence of alatae and apterae.

As referred to above, the alatae may be virginoparae or sexuparae in addition to males. Actually in nature, in England, the two latter forms do not occur until late September, or early October, a period coincident with a falling mean temperature, decreasing hours of daylight and scarcity of suitable food-plants (poor nutrition). The alate virginoparae which develop during the summer period are to be considered as dispersal forms, whose function is to ensure the distribution of the species. Under experimental conditions these forms may develop during the winter period, as will be described later, although, with moderate temperatures the alatae produced during that period tend to be sexuparae. The occurrence of alate sexuparae in autumn marks the appearance of the sexual phase, and the environmental factors concerned, as we shall see later, are correlated with rhythmical seasonal changes. These factors are (*a*) length of day, (*b*) temperature, and (*c*) plant growth (nutrition) as affected by (*a*) and (*b*).

(1) *Offspring of the alienicolae apterae.*

The offspring of the apterae may develop entirely into apterae or alatae or consist of a mixed brood of apterae and alatae. The alatae may consist of virginoparae, sexuparae and males. The two latter occur when the sexual phase is in evidence and all the alatae at this time may be

[1] Several species have been reared over long periods in a continuous parthenogenetic line.

(1) Slingerland (1893), according to Uichanco (1921), reared *M. persicae* for 62 generations over a period of 2 years 10 months.

(2) Ewing (1916) reared *A. avenae* for 87 consecutive parthenogenetic generations in California.

(3) Paddock (1919) reared *A. gossypii* for 51 generations.

(4) Comstock (*Introduction to Entomology*, 1924, p. 417) states that Slingerland carried on a species for 98 generations over a period of 4 years and 3 months.

(5) Reinhard (1927) reared *A. gossypii* for 59 generations in Texas.

(6) The writer reared *A. rumicis* through 50 generations (line B) in a period of 2 years and 10 months at Rothamsted. The number of generations passed through in a given time depends upon temperature and whether the first- or last-born young are selected to carry on the next generation.

sexuparae and males. The former occur when conditions favour parthenogenetic reproduction, when all the alatae may be virginoparae. During October and November, under natural conditions, the offspring of the apterae may develop entirely into alate sexuparae and males thus bringing parthenogenetic reproduction to an end. Under experimental conditions, the alatae which develop during the autumn and winter period may consist of a mixed brood of both sexuparae and virginoparae, or only sexuparae and males or only virginoparae depending on environmental factors, particularly temperature. It was found, for instance, that when a temperature about a mean of 70° F. was maintained, apterae predominated and the few alatae which developed were virginoparae. No case was recorded in which apterae produced sexual females as was observed by Shull(20) with *Mac. solanifolii*. The proportion of apterae which develop as offspring of apterae is affected by overcrowding, temperature and nutrition (physiological condition of food-plant). As referred to earlier, length of day (light factor) is also important in that it influences the occurrence of the sexual phase.

(2) *Offspring of the alienicolae alatae.*

The alate virginoparae are usually produced by apterae as explained in the previous sections. Their offspring usually develop into apterae, but alatae may also develop if the conditions are unfavourable, as for instance, when alatae are compelled to reproduce on plants which are heavily infested with aphids (overcrowding), or with poor nutrition. The offspring of alate virginoparae are, however, not so variable in this respect as is the case with the offspring of apterae. The alate sexuparae always appeared in the colonies about the end of September (except in line H) and afterwards in many generations throughout the winter period. They produced only sexual females, and no case was recorded in which alate sexuparae produced males as recorded by Shull(20) with *Mac. solanifolii*. No case was observed in which alatae produced both sexual females and virginoparae.

(3) *The effect of overcrowding on the occurrence of alatae and apterae.*

It has been frequently observed throughout these experiments, especially during the summer period, that, when a colony is started with one or two apterae, only an occasional alate form develops during the first 14 days or so, but alatae gradually become dominant as the colony increases, so that by the time the plant is heavily infested, large numbers of alatae are present.

In order to test whether overcrowding was a factor affecting this increase in the number of alatae, 15 bean plants, one in each pot (seed planted on the same day), were divided into three series of five plants each. The plants were infected with adult apterae from line I on 3. vi. 27, all being of the same generation. In series A one individual was transferred to each plant, in series B, five individuals and in series C, ten individuals. The plants were kept covered with muslin bags and reproduction allowed to go on until 20. vi. 27, when the aphids were killed off and the number of apterae and alatae on each plant counted. The results obtained are shown in Table III.

Table III.

Showing the effect of overcrowding on the occurrence of alatae.

	Number of aphids per plant			
		Alatae		
Series	Apterae	Adult	Nymphs	Alate %
A	61	2	0	
	59	0	0	
	52	0	0	1
	45	1	0	
	31	0	0	
B	244	39	38	
	208	41	45	
	179	90	46	34
	177	33	24	
	165	80	71	
C	449	108	121	
	363	133	89	
	358	48	46	39
	284	216	116	
	228	101	107	

The column, *apterae*, includes adults and immature individuals obviously going to develop into apterae. The column, *alatae nymphs*, includes individuals which possessed wing pads. Younger individuals were not counted, as alatae and apterae cannot be readily distinguished in the earlier instars. Many of these younger individuals would of course develop into alatae, as is shown by the results of two other series of five plants each, which were set up at the same time. Each plant was infected with three apterae and reproduction was allowed to go on for 5 days longer than in the case of Table III. The counts of the colonies showed the percentage of alatae in the two series to be 77 and 81 respectively. The influence of overcrowding is shown by the number of *adult* alatae present. In series C (Table III) an overcrowded condition occurred

earlier in the colonies, resulting in alatae developing early, many being adult when the colonies were killed off. In the two series in which the colonies were started with three apterae, the average number of adult alatae in each colony was only fourteen, but the number of alatae nymphs, compared with series A–C, was considerably increased as the longer reproduction period allowed more of the later-born individuals, born under crowded conditions in the colony, to attain the 3rd and 4th instar stage in which the wing pads are visible. During the summer period, if overcrowding in the colonies is prevented, the offspring of the alienicolae apterae develop into apterae, but as soon as overcrowding occurs, alatae tend to predominate. In the parthenogenetic line C, a succession of colonies were maintained on beans from the beginning of May until September, overcrowding being prevented by removing the adults from time to time. The results obtained are given in Table IV.

Table IV.

Showing offspring of apterae in successive colonies in the parthenogenetic line C when overcrowding was prevented.

E = *Euonymus*; M = Migrants; S = Sexuparae; V = Virginoparae.

Colony (beans)	Aphids transferred (1922)	Alatae and apterae in colonies after 10 days		
		Days	Apterae	Alatae
1 (E)	17·4	19	25	4 (M)
2 (M)	5·5	17	25	0
3	19·5	9	51	0
4	25·5	11	50	0
5	6·6	12	3	0
6	18·6	16	Few	0
7	5·7	14	Many	0
8	19·7	12	Many	0
9	5·8	25	Few	Few (V)
10	21·8	21	Few	Few (S)
11	11·9	39	2	6 (S) + 6 ♂

Colony No. 1 was started with one fundatrix on *Euonymus* and No. 2 with two alate migrants from No. 1. The remaining colonies were started with one or two apterae from the previous colony. Apterae predominated until about the middle of August, from which time onwards the colonies developed more slowly and a comparatively small number of aphids was produced. This was due to the seasonal conditions, chiefly falling temperature and poorer growth of the bean plants, together with the advent of the sexual phase. Under these conditions, alatae tended to predominate

in the colonies and those which developed in September were sexuparae. It should be noted that, although alatae were not present in colonies 2 to 8 at the end of the period of days shown in column three, nymphs began to appear a few days later and, as overcrowding increased, the number of alatae increased.

It is clear that overcrowding is an important factor affecting the occurrence of alatae in the summer period when factors of light, temperature and nutrition (food plants) are favourable. The influence of overcrowding may to some extent be interpreted as a nutrition factor in that, the young growth of the plant being crowded, the aphids are forced to feed on the older tissues, and the sap of the young growth affords the best nutrition. Further, as the infestation increases, the tissues of the plant are so affected that they do not function in a normal manner and the quality and quantity of sap available is affected. Overcrowding is, however, relative and the phenomenon is not only a matter of nutrition, as overcrowding may occur in a comparatively small colony on a local area of a plant, resulting in an increase of alatae.

(4) *The effect of nutrition on occurrence of alatae and apterae.*

The writer has shown(4) that the reproduction rate of *A. rumicis* varies on different food-plants and is also affected by the physiological condition of the plant(8). That the young growth of the bean plant affords the best nutrition for the insects is shown by the following experiment.

Two series, A and B, consisting of five bean plants each (seeds planted same day), were set up in pots. In series A the plants had normal growth, and in series B the young tops were cut off a few days before infection. On 31. v. 27 each plant in series A was infected with one adult apterous viviparous female from line I (offspring of alatae and reared to maturity on a normal bean plant). Similarly, each plant in series B was infected with one adult apterous viviparous female (offspring of alatae of same generation and reared to maturity on a bean plant having the top cut off). After 14 days' reproduction the ten plants were killed off and the aphids produced on each plant were counted. The results are shown in Table V.

It is evident that by removing the young top of the bean plant, its nutrition value for the aphis is affected. There is, however, no indication that the proportion of apterae and alatae has been influenced. It would be necessary to rear the aphids under these conditions through further generations to find out the cumulative effect, if any, of the two sets of

Table V.

Showing the comparative reproduction rate on beans with normal growth (A) and with tops cut off (B).

Series	Apterae	Alatae	Total aphids present	Mean
A	44	0	390	
	34	0	276	
	33	0	384	335·2
	32	0	339	
	29	5 (N)	287	
B	24	1 (N)	163	
	24	0	165	
	23	0	84	104·8
	14	0	72	
	10	0	40	

N = Nymphs.

conditions. Data available from experiments made to test the influence of different food plants and of the physiological condition of the bean plant on the reproduction rate of the bean aphis indicate that, on those plants which favour a high reproduction rate, the proportion of alatae which develop is smaller than on those plants on which a low reproduction rate occurs. This was observed, for instance, with poppies, peas, turnips and mangolds compared with broad beans, and its occurrence on *Euonymus* has been already referred to. Similar observations were made with different varieties of field beans. The available data does not allow of definite conclusions being drawn, but the factor of overcrowding does not appear to be the only one concerned, and the question as to whether the nutrition value of different food-plants affects the proportion of alatae and apterae which may develop requires further investigation. There is no doubt that the physiological condition of the food plant, in that it affects the nutrition of the insect, is a factor of importance, which must be considered when the influence of other external factors are being investigated if uniform results are to be obtained. The starvation experiments of Gregory (12) and Wadley (23) show clearly that poor nutrition of the parent female results in an increase in the proportion of alatae in the offspring.

During the winter period in the heated glasshouse, beans grow spindly and are poor plants compared with those grown in the summer months, due chiefly to the effect of temperature in relation to the winter light conditions. The aphid colonies on these winter plants progress more slowly than in summer, even when summer temperatures are maintained. The developmental period of individuals in winter, when reared under

"summer" temperatures approximated closely to that obtained with summer individuals (effect of temperature), but the aphids individually were not so prolific. Moreover, while with a mean temperature of about 60° F. in winter fewer apterae were obtained, apterae predominated under these temperatures during the summer period if overcrowding was avoided, which suggests the influence of the better nutrition value of the plants in summer. During the winter period with moderate temperatures alatae tended to predominate in the colonies, although with comparatively high temperatures (see p. 121) the influence of the nutrition factor is overcome and apterae predominate. It is interesting to note that the apterae frequently feed below the leaves on these winter plants and not on the growing apex, whereas in summer they invariably feed on the growing tip, unless overcrowded conditions force them to the older parts of the stem and beneath the older leaves. On two or three occasions during the winter period, it was observed that the aphids on bean plants which became sickly owing to root rot developing, left the plant (particularly the apex of the stem) and wandered to the muslin covers. These observations show that the aphids react to the physiological condition of the plant.

From March onwards there is a marked improvement in the growth of the bean plants compared with the earlier period of winter, and as spring advances, if a favourable temperature is maintained, the aphid colonies make better progress and a higher proportion of apterae develop.

(5) *The effect of temperature on occurrence of alatae and apterae.*

It has been shown that during the summer period when the aphids are reared on a favourable food plant (broad beans) and overcrowding avoided, the offspring of apterae tend to be predominantly apterae. Favourable nutrition, correlated with the large area of succulent growth on the plants, is the most important factor favouring the occurrence of apterae during this period, and the moderate fluctuations in the summer temperature do not markedly influence the sequence of alatae and apterae. Alatae, however, predominate irrespective of temperature when overcrowding occurs. During September, under the influence of a falling temperature, shorter hours of daylight, and lack of suitable food-plants (nutrition), the aphid colonies are much smaller and alatae tend to predominate even when overcrowding does not occur and comparatively few apterae may develop. When the aphids were transferred to a warm glasshouse in October, the proportion of apterae increased. In Fig. 3 the results are shown of counts made of apterae and alatae

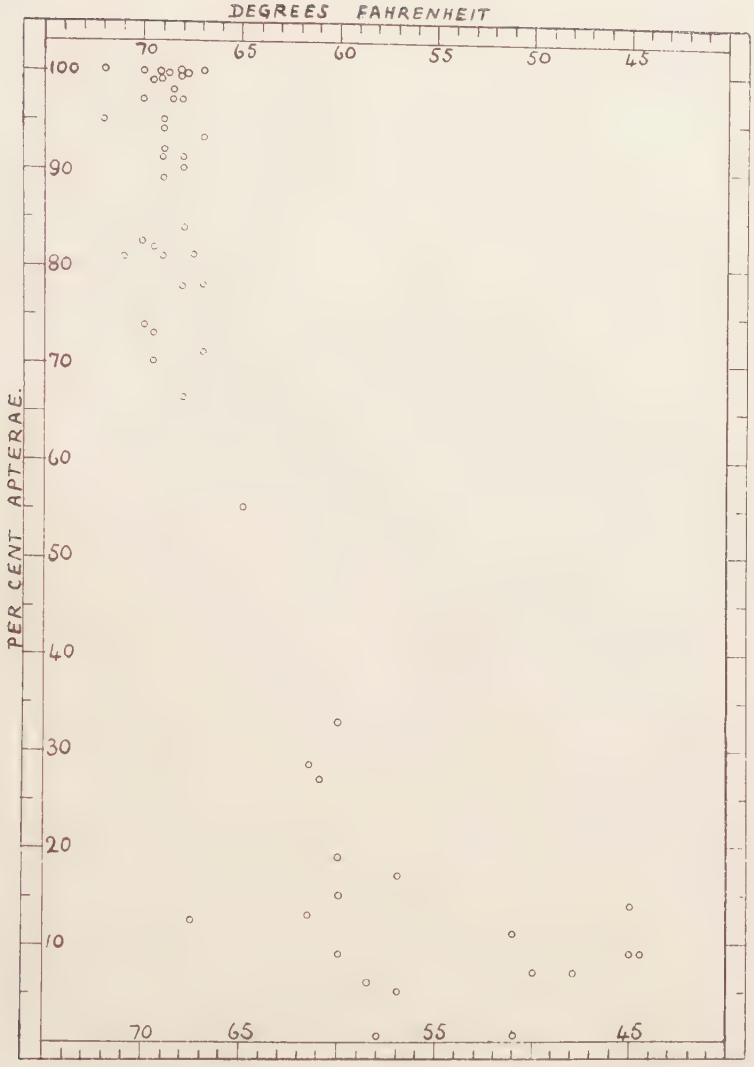


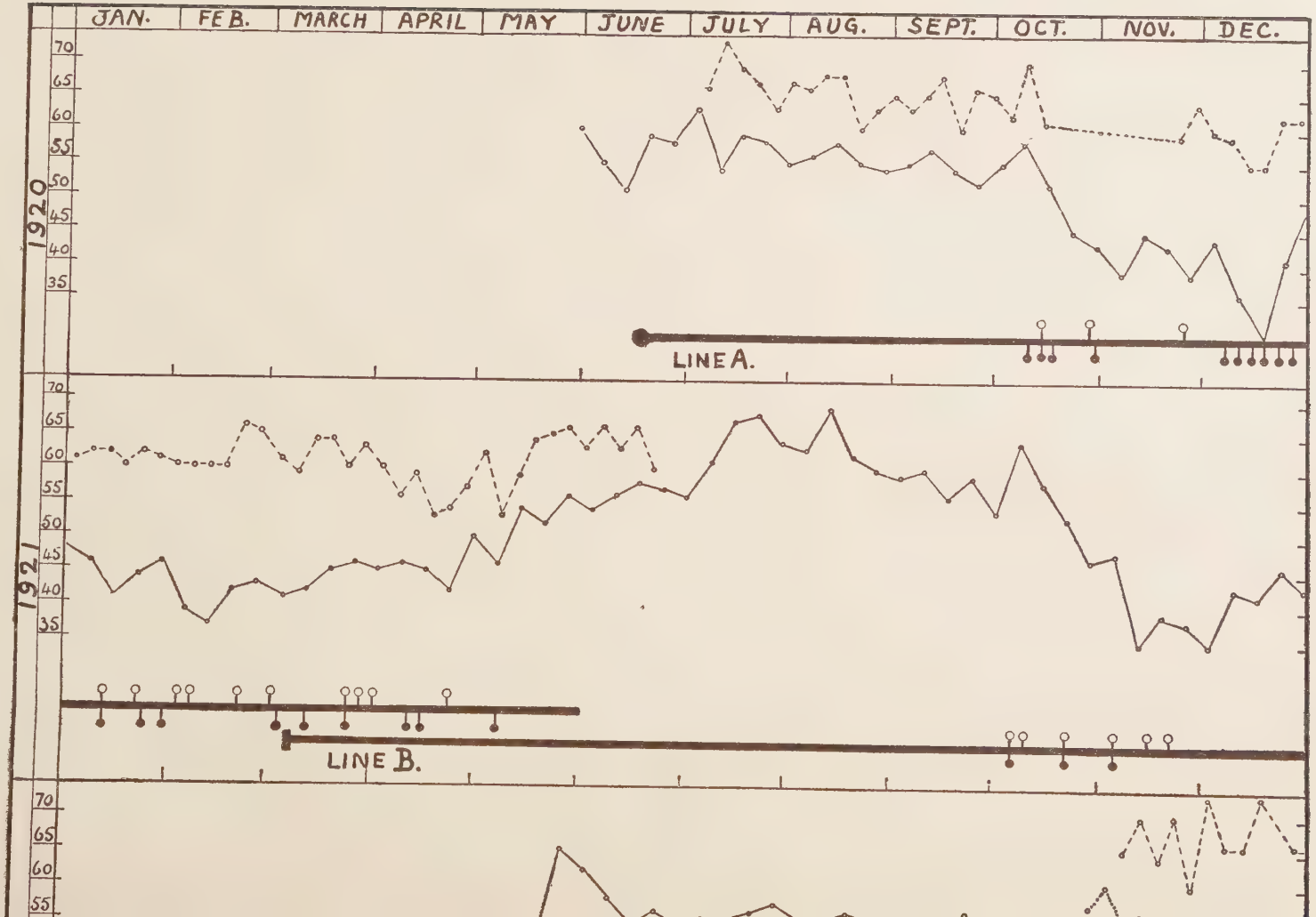
Fig. 3. Showing the influence of temperature on the occurrence of apterae and alatae during the winter period. Counts were made from 56 colonies (offspring of apterae) in lines A, B, C, G, H, Ha during the winter period and the percentage of apterae present in each colony is plotted with reference to the mean temperature of the reproduction period in each case. As far as possible precautions were taken to prevent overcrowding.

present in 56 colonies reared under various temperatures during the winter period. The colonies were taken from five of the parthenogenetic lines in different years. They were killed off for counting after a reproduction period of 2 to 3 weeks or longer in the case of the lower temperatures. Precautions were taken to prevent overcrowding and the alatae and apterae were diagnosed as in the experiments referred to in a previous section. With a mean temperature of about 67° F. and over there was a marked increase in the proportion of apterae in the colonies, and below about 57° F. there was a relatively small proportion of apterae. It should be noted that these counts include the colonies which received artificial light, as there was no apparent difference in the proportion of apterae and alatae present compared with the control colonies, as is seen in Table VI. The temperatures maintained in both these sets of experiments were moderately high.

B. Occurrence of the sexual forms.

Owing to the large number of colonies which were reared in the various parthenogenetic lines, it is not feasible to present the data in the form of tables, but in Figs. 4 and 5 the occurrence of the sexual forms has been indicated by symbols placed in positions which show the approximate dates when *adult* sexual individuals were recorded in the colonies. Each symbol represents a varying number of individuals present in a colony, so that usually the total number of symbols on any parthenogenetic line shows the number of colonies in which sexual forms were recorded.

It will be seen from Fig. 1 that, with the exception of line H in which the colonies were reared under shorter hours of daylight, *adult* sexual forms occurred with great regularity each year in October, and where the lines were continued parthenogenetically throughout the winter, they appeared from time to time from October until about the end of the following May. It is interesting to note that, although sexual forms developed during the spring and early summer months in overwintered lines, they did not develop in the colonies of lines started from the fundatrix in spring until the following October, although during the spring period both sets of colonies were reared under the same conditions. In the colonies of the overwintered lines there was, however, a progressive increase in the proportion of parthenogenetic individuals present in the colonies as spring advanced, compared with the earlier winter period: alate virginoparae began to appear during March and April, the number of alate sexuparae becoming less, so that about the end of May, the alate forms tended to be entirely virginoparae. The occurrence of the sexuales


JAN.
FEB.
MARCH
APRIL
MAY
JUNE
JULY
AUG.
SEPT.
OCT.
NOV.
DEC.
1920
70
65
60
55
50
45
40
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LINE A.
1921
70
65
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55
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LINE B.
70
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55

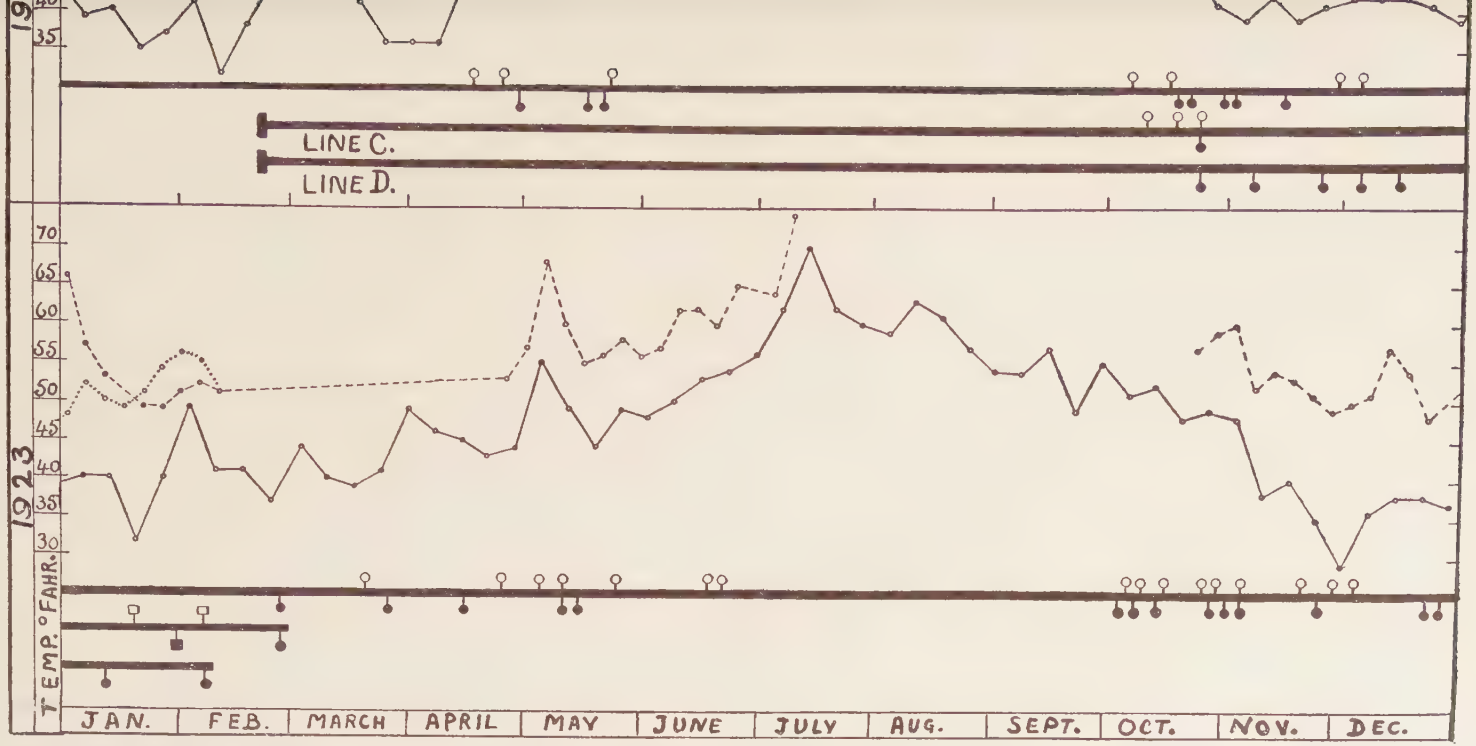


Fig. 4. The chart shows dates on which adult sexual forms of *A. rumicis* were recorded in the colonies of the parthenogenetic lines A–D. The thick horizontal lines show the length of time parthenogenetic reproduction was maintained in each of these parthenogenetic lines. The symbols attached along these lines represent adult males (small open circles above the lines) and adult sexual females (small closed circles below the lines). The places where these symbols are attached indicate the approximate dates these forms were recorded in the colonies. It will be noted that during June to September only parthenogenetic individuals were obtained.

The air temperature at Rothamsted (daily mean of weekly periods) is shown by the whole-line curve; the glasshouse temperature at different periods (daily mean of 5-day periods) is shown by a broken-line curve. During 12. xi. 22 to 12. i. 23 the colonies in lines B and C received artificial light (note absence of sexuales) and temperature as shown in upper curve (broken line). With line C (January 1927) the square symbols indicate occurrence of sexuales in colonies receiving same temperature, but no artificial light. With line D the colonies received only ordinary daylight during this same periods, and lower temperatures as shown in middle (dotted line) curve (note occurrence of sexuales).

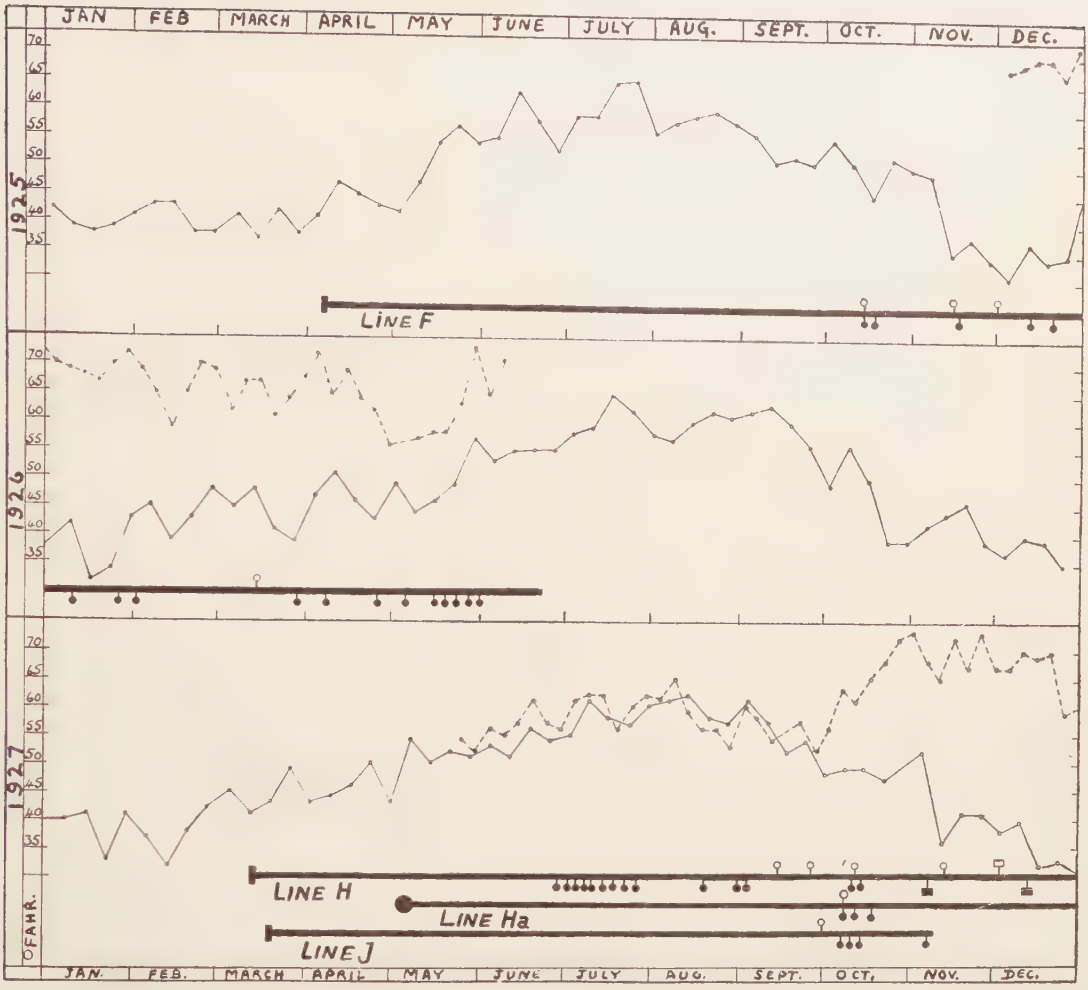


Fig. 5. The plan of this chart which shows the data obtained with the parthenogenetic lines F, H, Ha and I is as described for Fig. 4. The whole-line temperature curve shows Rothamsted air temperature. The broken-line temperature curve in 1927 refers to the open-air insectary (up to 30. ix. 27) and after that date the heated glasshouse.

During the period 28. iii. 27 to 5. ix. 27 the colonies in line H received only 8 hours daylight daily (note early appearance of sexual females). During period 25. x to 23. xii. 27 the colonies in line H and Ha received artificial light in excess of normal daylight (note absence of sexuales): the square symbols indicate occurrence of sexual forms in colonies not receiving artificial light.

From particulars given in Section II of the paper, by referring to Figs. 4 and 5, the reader can see the conditions under which the aphids were reared throughout the year.

Charts IV and V should be compared with Fig. 1.

during the spring period indicates a carrying-over effect of the previous winter conditions under which the aphids were reared.

After the sexual forms first began to appear in the various lines, apterae were transferred in successive generations to form new colonies and so continue the parthenogenetic line: the alate offspring were tested out in succeeding generations to see whether they produced sexual females (being sexuparae) or parthenogenetic females (being alate virginoparae). It is necessary in order to make sure that the sexual phase is present or absent, to rear the offspring of these alatae in as many of the successive generations as possible, in order to test whether the latter are sexuparae or virginoparae. In my experiments males were found sometimes to occur somewhat irregularly during the winter period, and one cannot rely upon their non-occurrence as indicating that the sexual phase is absent. Moreover, under the higher temperature conditions during the winter period many alatae died without reproducing, and it was necessary to isolate these forms carefully so as to ensure that they would reproduce. It is due to the precautions taken in this way and to the fact that by rearing a large number of colonies, a representative number of the insects in successive generations were available, that the presence of sexual forms has been demonstrated over such extended periods.

The details regarding the occurrence of the sexuales in the various lines are given below; the dates given are those on which the sexual forms were adult.

1. *Line A.* The sexual phase set in early in October 1920. From October until the following May, 37 colonies were reared and adult sexual forms occurred in most of them on various dates as shown in Fig. 4. After May 1921 no sexual forms occurred.

Males were first recorded on 14. x. 20 and developed in 12 later colonies, the last record being 23. iv. 21. Altogether about 50 males were recorded.

Females were first recorded on 14. x. 20. They appeared in 21 later colonies on various dates, the last date being 7. v. 21. Throughout the winter period, the alatae tested out proved to be only sexuparae, but on 23. iv. 21 both sexuparae and alate virginoparae were present and after this date only virginoparae developed.

2. *Line B.* As this line was carried on through three consecutive winter periods, it will be more convenient to deal with these three periods separately. It will be noted from Fig. 1 that there was a rhythmical

appearance of the sexual forms in October each year and a suppression of the sexual phase from about the end of May onwards, until the following October.

First winter period 1921–1922.

The sexual forms appeared early in October 1921 and on various dates until the following May. The temperature in the glasshouse during the greater part of this winter period was comparatively low (about a mean of 50° F.) so that the developmental period of the aphids was long and only 12 small colonies were reared. This explains the few records for sexual forms which were obtained, but the sexual phase was in evidence throughout the period.

Males were first recorded on 6. x. 21 and they occurred in eight subsequent colonies, the last record being 23. v. 22. About 24 individuals were recorded.

Females were first recorded on 6. x. 21 and they developed in five subsequent colonies, the last record being 18. v. 22.

Second winter period 1922–1923.

Sexual forms again appeared in October 1922, being present in six colonies during this month and the early part of November. During the period November 8th, 1922 to January 12th, 1923, high temperatures were maintained (see Fig. 4), and the successive colonies (ten in number) received artificial light as explained in Section II. Under these conditions parthenogenetic reproduction was vigorous, apterae predominated and comparatively few alatae developed. Of the latter, some were tested out on 6. xii. 22 and 20. xii. 22 and were found to be virginoparae. With the exception of one male which developed on 29. xi. 22 and one on 6. xii. 22, no sexual forms occurred in these ten colonies.

Five control colonies reared during the same period, under the same temperature, but without artificial light, behaved similarly and no sexual forms developed in them. The results obtained with these control colonies are given in Table VI, together with those of six of the colonies which received artificial light, to show the number of individuals produced under these conditions. These data have been included in Fig. 3.

Five apterae were isolated from one of these control colonies on 6. xii. 22 and placed under lower temperature (middle dotted line in Fig. 4). The majority of their offspring developed into alate sexuparae. It should be noted that even with high temperatures during the winter period—which favours parthenogenetic reproduction—the winter light

Table VI.

Showing the proportion of apterae produced in colonies of line B during winter period 1922–1923.

A.L. = artificial light series; N.L. = controls with no artificial light.

Colony no.		Infection with apterae	Reproduction period days	Mean temp. of period ° Fahr.	No. of aphids		Apterae %
A.L.	N.L.				Apterae	Alatae	
51	—	2	21	67	32	0	100
59	—	2	14	68	52	8 (N)	88·6
—	48	2	14	68	20	2 (N)	91
—	54	2	14	68	10	0	100
45	—	5	14	68	40	5	89
60	—	2	14	68	44	0	100
—	49	2	14	68	35	1	97
—	55	2	21	67	25	2	93
52	—	2	21	69	86	8 (N)	91·4
61	—	2	14	67	48	0	100
—	50	2	14	67	20	8 (N)	71·4

conditions tend to exert an influence in favouring the occurrence of the sexual phase as will be discussed later.

After the middle of January 1923 the aphids in line B were reared under lower temperatures. From the middle of January until June 1923, eleven colonies were reared in which males developed on various dates, the first being recorded on 20. iii. 23, the last record being 22. vi. 23. Altogether about 26 males were recorded in this second winter period. Females also developed in five of the eleven colonies referred to above, the first record being 28. ii. 23 and the last 15. v. 23. The alatae tested out after 24. v. 23 proved to be only virginoparae and no sexual forms occurred in the colonies until the following October (third winter period).

Third winter period 1923.

Males were first recorded on 6. x. 23 and they appeared in seven further colonies on various dates, about 47 individuals being recorded. Females were first recorded on 3. x. 23 and subsequently in six further colonies. The parthenogenetic line was discontinued at the end of December 1923. It will be noted (Fig. 4) that the temperature conditions during this winter period were more favourable for the occurrence of sexual forms than was the case with the somewhat higher temperatures maintained during November and December of the previous winter.

3. *Line C.* Sexual forms appeared in this line in October 1922. During the period November 8th 1922 to January 12th 1923 five

colonies were reared under similar temperatures and artificial light as in line B. The results obtained were similar, sexual forms did not develop, but females occurred later in a continuation colony from this series on 28. ii. 23. Six control colonies were reared during this period under similar conditions but without artificial light, and no sexual forms occurred. However, they appeared earlier in the colonies descended from this control series than in the colonies descended from the series which received artificial light. One male developed on 19. i. 23 and another on 7. ii. 23 and females developed on 31. i. 23. These cases are shown in Fig. 4 by square symbols. Altogether 8 males were recorded in line C. The parthenogenetic line was discontinued at the end of February 1923.

4. *Line D.* In this line adult females were first recorded on 24. x. 22 and they developed in five subsequent colonies, the last record being 8. ii. 23. No males were recorded which is probably due to the fact that only a few colonies were reared and the number of aphids produced was small owing to the lower temperatures (see Fig. 4, middle dotted line). It is interesting to note that, under these lower temperatures, the alatae which developed were only sexuparae and sexual females were produced throughout the period.

5. *Line E.* Males and females were recorded in October and November 1924, and the parthenogenetic line was discontinued at the end of the latter month.

6. *Line F.* Sexual forms appeared about the middle of October 1925 and occurred during November. From 1. xii. 25 onwards throughout the winter period, twenty-six colonies were reared, and sexual females developed at frequent intervals as shown in Fig. 4, the last record being 30. v. 26. The alatae tested out from time to time proved to be sexuparae, but in two colonies on 8. iv. 26 and 29. iv. 26 alate virginoparae were also present and after 17. v. 26 only virginoparae developed, the sexual phase being suppressed. It was particularly noted in this line, during the period December to March, that many alatae died without reproducing, and careful attention was necessary in order to get the alatae to reproduce. In one instance, about 14. xii. 25, one plant was infected four times with a total of 32 alate forms and only 4 individuals were produced, which were sexual females. The temperature conditions evidently favoured parthenogenetic reproduction and the sexual forms obtained were few in number. Even where sexuparae developed, they were induced to reproduce only with difficulty. From December 1st onwards two isolated males were obtained, one on 2. xii. 25 and the other on 15. iii. 26.

It will be noted that the temperature during this winter period was only slightly lower than during the period November 1922 to January 1923, when, in lines B and C, the sexual phase was practically suppressed in the control colonies and completely suppressed in those colonies which received artificial light.

7. *Line G.* Sexual forms appeared in October 1926 and the parthenogenetic line was discontinued early in November.

8. *Line H.* Under the conditions of short hours of daylight in this line (see Section II) adult sexual females were first recorded on 29. vi. 27. From 18. vi. 27 to the beginning of October, eighteen colonies were reared and females occurred in thirteen of these, the last record being 15. x. 27. It was noted that from the beginning of June the offspring of the apterae tended to be alatae, which proved to be sexuparae. Males, on the other hand, were not recorded until 14. ix. 27, that is, about the normal time, others were recorded on 26. ix. 27 and 15. x. 27, in all a total of five individuals.

From October 6th onwards eight colonies were reared under higher temperatures and received artificial light as stated in Section II. Under these conditions parthenogenetic reproduction was vigorous and the aphids which developed were in a large majority apterae. The comparatively few alatae which developed were found to be virginoparae, and no sexual forms were obtained. Nine control colonies were also reared under the same temperatures, but without artificial light. Apterae predominated also in these control colonies and comparatively few alatae developed; those tested out on 15. x and 2. xii. 27 proved, however, to be sexuparae and sexual females developed on 5. xi and 13. xii. 27. One male was recorded on 2. xii. 27. These three instances are shown in Fig. 4 by means of square symbols.

These results support those obtained under similar conditions during November and December 1922 in line B. The evidence from line H shows that favourable temperatures and long hours of daylight favour parthenogenetic reproduction, whereas the reverse conditions favour the sexual phase. Even with favourable high temperatures during the winter period (short hours of daylight), although the temperature favours parthenogenetic reproduction, there is a tendency for the sexual phase to develop, presumably due to the light factor, as is seen in the behaviour of the control colonies in this line.

9. *Line Ha.* This is a control for line H, the aphids being descended from the same fundatrix. Sexual forms did not develop in the colonies

until the middle of October 1927. Females were recorded in three colonies on October 8th, 10th and 17th, and the alatae tested out proved to be only sexuparae. Three males occurred in one colony on 17. x. 27. Colonies from this line were established on *Euonymus*, ova were laid and fundatrices commenced to hatch out on 25. iii. 28.

From the middle of October to the end of December, ten colonies were reared which received artificial light as in line H. The aphids in these colonies were chiefly apterae, very few alatae developed and those tested out on 16. xi, 12. xii and 15. xii. 27 proved to be virginoparae. No sexual forms were obtained.

Eight control colonies were reared during the same period as in line H. Parthenogenetic reproduction was vigorous and no sexual forms were obtained.

10. *Line I.* In this control line sexual forms did not occur until October, females being recorded in four colonies about 10. x. 27 and 14. xi. 27. Three males developed in one colony on 30. ix. 27. The line was discontinued about the middle of November.

VII. GENERAL CONCLUSIONS AND SUMMARY.

1. *Material employed in the experiments.*

Ten related lines of the black bean aphis (*Aphis fabae* Scop. = *A. rumicis* L.), in which continuous parthenogenetic reproduction was maintained for varying periods, have been reared during the past seven years (Fig. 1). The longest period was 2 years 10 months with line B, 50 generations having been passed through. The lines were all of the same strain, nine of them being descended from line B, which was started from one fertilised egg. The remaining lines (except Ha) were similarly started from fertilised eggs.

Euonymus europaeus was used as the primary food-plant (winter host) and broad beans as the intermediate (summer) food-plant. During the summer period (April to October) the aphids were reared in a large open glasshouse or in the open-air insectary, and during winter (November to March) in a heated glasshouse with varying temperatures (Figs. 4 and 5). The *Euonymus* plants on which fertilised eggs were laid in autumn were kept outside in the open during the winter period.

2. *The normal life cycle of* Aphis rumicis *L.*

Aphis rumicis is a migrating species in England, its usual winter host being *Euonymus europaeus*. Its normal life cycle resembles that of other migrating Aphidini; sexual forms occur about October and the fertilised eggs commence to hatch the following March (Fig. 2).

3. *Fundatrigeniae generations.*

The fundatrix is considered in this paper as the first generation. Its offspring are usually apterae but may also include alatae (migrants). The apterae of second generation give rise chiefly to alate migrants and a few apterae. The offspring of the latter forms normally consist of alate migrants so that the fundatrigeniae generations come to an end.

There is an inherent tendency for the offspring of the fundatrigeniae apterae to develop into alate migrants, evidently associated with the evolution of the migrating habit. The proportion of alatae and apterae is, however, influenced by environmental factors, particularly overcrowding and the condition of the food-plant (nutrition)[1].

4. *Alienicolae generations.*

The alate migrants have a strong inherent tendency to migrate from the primary host plant, but if confined on *Euonymus* they reluctantly reproduce on it. Moreover, while their offspring on beans develop into apterae, when produced on *Euonymus* some of them develop into alatae owing to the influence of the nutrition factor. The migrants initiate the alienicolae generations. Under certain environmental conditions the succeeding alienicolae generations may consist only of parthenogenetic individuals, namely apterae and alate virginoparae. Under other conditions sexual forms develop. The latter consist of alate males (offspring of apterae) and apterous females (offspring of alate sexuparae). The proportion of sexual and parthenogenetic individuals which develop depends upon environmental factors: on the one hand we may get the parthenogenetic generations terminated owing to the fact that sexual individuals only are produced: on the other hand, only parthenogenetic individuals may be produced. Conditions may occur under which both types are represented.

[1] In non-migrating species, migrantes in the true sense do not occur and the alatae are to be considered as dispersal forms. Reinhard (19) considers that with *Aphis gossypii* the normal tendency is for the parthenogenetic individuals to be apterous. Baker and Turner (2) showed that with *Aphis pomi* the complete bi-sexual cycle could be completed without the occurrence of alate individuals.

A. The occurrence of alatae and apterae.

Three types of alate parthenogenetic females occur during the complete life cycle, namely (1) migrantes, (2) dispersal forms, (3) sexuparae. These three forms resemble one another morphologically, but they differ in that each type plays a different rôle in relation to the migrating habits of the insect. It is necessary therefore to consider them separately when dealing with the influence of various factors on the occurrence of apterae and alatae.

The migrantes have been dealt with in the previous section as they belong to the fundatrigeniae generations. The dispersal forms and the sexuparae occur in the alienicolae generations.

The alate dispersal forms (alate virginoparae as distinct from the alate sexuparae) are chiefly offspring of apterae. They ensure a wide distribution of the species, but are not essential for the completion of the bi-sexual cycle. Their occurrence depends upon environmental factors, particularly (1) overcrowding, (2) temperature, (3) nutrition. Correlated with their function as "colonisers," their offspring normally develop into apterae which latter are more prolific than the alate forms. Under favourable conditions the offspring of apterae develop into apterae, but with adverse conditions of overcrowding, low temperatures and poor nutrition, alatae may also develop. The proportion of apterae and alatae depends upon the influence of these three factors. Actually during the summer, temperature does not play such an important part owing to the moderate fluctuations about a favourable mean temperature.

The experimental results obtained by Wadley(23) and others with other species of aphids show that under the influence of one or more of the factors referred to above, the offspring of apterae (and to some extent alatae) may develop into apterae or alatae. The fate of the individuals in this respect may be determined in the early instars immediately after birth, or during pre-natal development in the parent female. From the embryological studies of Uichanco(21) we know that the embryos of the young offspring are well advanced, even in the early instars of the parent female. With the exception of the work of Ackerman(1) no detailed investigations have been made on the physiological aspect of the occurrence of apterae and alatae, and it is to be hoped that his interesting observations will be studied further.

The alate sexuparae mark the appearance of the sexual phase. The occurrence of the latter is associated with the seasonal factors of light (length of day) and temperature.

B. The occurrence of the sexuales.

The rhythmical occurrence of the sexuales in October each year in the various lines (Figs. 4 and 5), indicates that this phenomenon is due to the influence of seasonal factors. Light (length of day) and temperature have been shown to be the important factors. While with normal daylight adult sexuales first occur in October, it was found that in the case of line H, in which the colonies received only 8 hours daylight daily, sexual females first appeared in June and were obtained in subsequent generations throughout the summer.

When the various lines were continued parthenogenetically throughout the winter in a heated glasshouse using normal daylight, sexual forms appeared in the colonies until the following May, provided a moderate temperature (about a mean of 58° F.) was maintained (lines A and D). From March onwards the proportion of parthenogenetic individuals increased and with the exception of two instances in early June, adult sexuales were not obtained during June to September (excluding line H), but they reappeared again in October (line B).

With moderately high temperatures during the winter period (about a mean of 70° F.) sexual forms were obtained only rarely or not at all (lines B, H, indicated by square symbols). There was a high proportion of parthenogenetic individuals under these conditions and apterae predominated.

With slightly lower temperatures during the winter period (line F) a few sexual females were obtained in most generations, but the alate sexuparae did not readily reproduce. Males were rare.

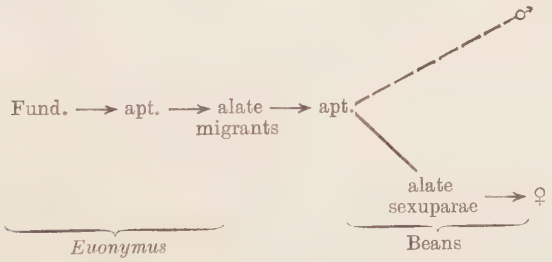
When the colonies were subjected to artificial light in excess of the normal daylight during the winter period, and a mean temperature of about 70° F. maintained, no sexual forms appeared (lines B and C, November 1922 to January 1923 and lines H and Ha, October to December 1927).

The higher temperatures favour the development of parthenogenetic forms, while the winter light conditions favour the development of the sexual forms. The normal parthenogenetic method of reproduction during the summer, and the suppression of the sexual phase is to be considered as an expression of the influence of these favourable seasonal factors[1].

[1] The few records of the occurrence of sexuales in tropical and sub-tropical countries does not necessarily indicate a complete absence of these forms in most species. Owing to the favourable environment, parthenogenetic forms may be dominant, and the sexuales comparatively few. Yingling (*Journ. Econ. Entom.* p. 223, 1917) records the sexual females and eggs of *Anoecia corni* in South Texas during December.

From the cytological studies of Morgan, von Baehr, and others it is evident that the mechanism which determines the change from the parthenogenetic to the sexual individual, is associated with the chromosomes. The fate of the offspring of the sexuparae is therefore determined during their pre-natal development. Thus, for instance, although adult sexual forms occurred up to about the end of May, they were probably determined by the conditions obtaining about the end of April. When adult alate sexuparae were isolated in July from colonies in line H and placed under normal daylight conditions, their offspring developed into sexual females. Similarly, during the winter period, when alate sexuparae, reared under temperatures about 58° F., were transferred to higher temperatures, their offspring developed into sexual females.

The fundatrix is also predetermined in the fertilised egg, and Baker and Turner(2) have shown with *Aphis pomi* that the embryo is well advanced by the time the fertilised egg assumes the winter resting condition. With migrating species of aphids like *A. rumicis* it is possible that not only is the fundatrix predetermined but also the occurrence of the alate migrants is inherently established. This would explain why sexual forms did not appear in line H until June. Actually in this instance alate sexuparae developed in the second alienicolae generation as shown below.



Normally, in nature, many alienicolae generations are passed through before the sexuales appear in autumn, and the results referred to above show clearly that the sexual phase develops irrespective of the number of generations passed through. The seasonal factors concerned in affecting the occurrence of the sexual and parthenogenetic phases in the life cycle of *A. rumicis* are shown graphically in Fig. 6, and the dates of the

occurrence of sexual forms in the various lines should be referred to this diagram.

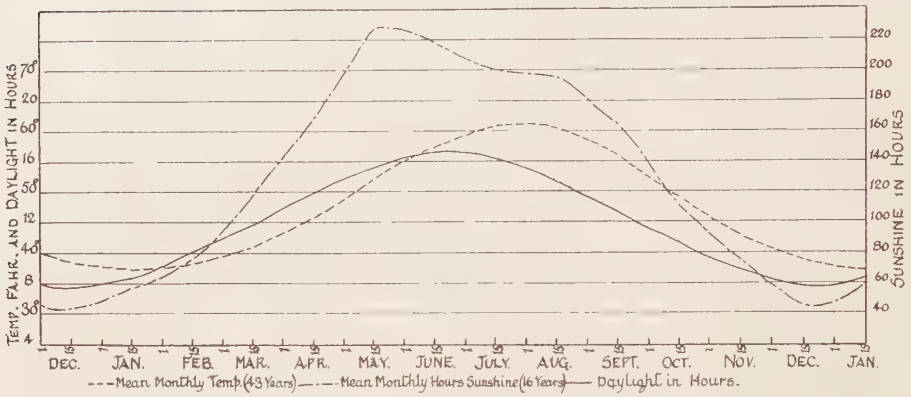


Fig. 6. Diagram illustrating the relation of seasonal factors of temperature and sunshine (Rothamsted Records) and daylight throughout the year. The dates of the occurrence of sexual forms in the various lines of *A. rumicis* should be referred to this diagram.

SUMMARY.

1. Ten related lines of *Aphis rumicis* L. have been reared in which parthenogenetic individuals were obtained in successive generations extending over varying periods (Fig. 1): the longest period was 2 years 10 months (line B), 50 generations having been passed through.

2. The aphids were reared in an open-air insectary or a large, open glasshouse during April to October (summer period) and in a heated glasshouse with varying temperatures (Figs. 4 and 5) during November to March (winter period): *Euonymus europaeus* was used as the primary host and *Vicia faba* as the intermediate food plant.

3. The life cycle of this species resembles that of the normal type of migrating Aphidini: in S.E. England the fundatrix usually hatches in March and the adult sexuales appear in October.

4. Three types of alate parthenogenetic females occur during the complete cycle, namely migrantes, dispersal forms and sexuparae: these forms resemble each other morphologically but differ in their relation to the migrating habits.

(*a*) The migrantes may develop as offspring of the fundatrix, but more usually they are offspring of the fundatrigeniae apterae: their occurrence is due to some extent to an inherent established tendency, associated with the migrating habit, but the numbers which may occur in the various fundatrigeniae generations is influenced by overcrowding

and the condition of the food plant (nutrition): they normally produce apterae (alienicolae).

(*b*) The alate dispersal forms are usually offspring of alienicolae apterae, but may also be produced by the alatae: their occurrence in the various alienicolae generations is affected by overcrowding, nutrition and temperature: they normally produce apterae, but under adverse conditions may also produce alatae.

(*c*) The alate sexuparae are offspring of alienicolae apterae and their occurrence marks the appearance of the sexual phase; they produce sexual females.

5. With suitable environmental conditions the parthenogenetic alienicolae generations may be maintained experimentally for long periods, and the sexual phase may be induced or suppressed depending on the factors of light (length of day) and temperature. The effect of nutrition (if any) due to the influence of these factors on the plant has not been determined.

6. With the normal seasonal hours of daylight, adult sexual forms appeared regularly in the various lines in October each year. Moreover, they were obtained throughout the winter period until the following May when moderate mean temperatures (about 58° F.) were maintained (lines A and D); with higher mean temperatures (about 70° F.) during this period, they only occurred occasionally (lines B and C, November 1922 to January 1923, and lines H and Ha, November to December 1927); with slightly lower mean temperatures (line F) a few sexual females were obtained in every generation, but males were rare.

7. When the colonies received only 8 hours daylight daily from the fundatrix stage onwards (line H), sexual females developed in June and subsequent months; males did not appear until October.

8. When colonies received artificial light from electric lamps, in addition to the normal hours of daylight, during the winter period (lines B and C, November 1922 to January 1923, and lines H and Ha, November to December 1927) no sexual forms were obtained; the mean temperature was about 70° F. and, as stated in paragraph 6, the sexual forms only occurred in isolated instances in the control plants (received no artificial light) owing to the influence of the high temperature.

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STUDIES ON *OSCINELLA FRIT* LINN.

A REPORT ON CERTAIN OAT VARIETIES IN RELATION TO THEIR RESISTANCE TO ATTACK BY THE FRIT FLY IN SWEDEN, TOGETHER WITH DATA CONCERNING THE PRODUCTION OF RESISTANT UTILITY VARIETIES

By NORMAN CUNLIFFE, M.A.

(*Research Officer, School of Rural Economy, University of Oxford.*)

(With 8 Charts.)

CONTENTS.

I. INTRODUCTION.

Methods of control most generally advocated are palliative in nature, are primarily adapted for crops cultivated intensively (which permit expenditure on a comparatively high scale) and can only be advocated for these types of crops. The protection of farm crops, produced under extensive systems of cultivation, has been neglected partly because of the adverse economic factor and partly because of the difficulties to be surmounted in exploring any but the direct methods of control. Non-acceptance of the view that insects can be controlled only by direct

methods in practice necessitates a wider outlook on the subject and, consequently, the exploration of the possibilities of indirect methods of control, other than the well-known biological method. As it is, at present, impossible to move the prevalent periods of a pest in time, we must necessarily endeavour either to control the susceptible period of plant growth or to aim at the production of resistant or immune varieties. Preliminary studies of this nature, revolving round the resistance of a plant to infestation, immediately and most strongly emphasise the primary importance of the conception of the plant as the central figure and indicate that, in spite of the urgency of special economic problems, the more distant problems associated with the growth phases of the plants, namely, resistance to infestation, recovery power after injury, etc., must be explored if marked and permanent progress is to be expected.

In this report is described research work which was undertaken by means of a Travelling Research Fellowship in Agricultural Entomology, awarded in 1927 by the Ministry of Agriculture and Fisheries. The investigation was conducted at Sveriges Föreningen (The Swedish Seed Institute), situated in the village of Svalöv, in the southern Swedish province of Skåne. The object of this investigation may with advantage be stated briefly at once. At Svalöv, claims have been made that varieties of oats, which have shown marked powers of resistance to attack by the frit fly, have been and are being established. If these claims could be substantiated then (1) it was urgently necessary to have first-hand knowledge of the factors involved in order to determine whether similar strains or varieties could be utilised in England, and (2) it was equally necessary to study the methods in use at Svalöv in connection with oat breeding, especially in relation to resistance to pests and diseases. On the other hand, if such claims were found to be unsupported by substantial scientific observation, this knowledge in itself would be of considerable importance in England, because we should then be in a position to judge such claims and therefore know to what degree we might expect new varieties to be of value to the English agriculturist.

It is to be regretted that the spring season was the worst experienced in South Sweden since about 1880 and the growing season generally the least favourable for the last twenty years, because these very adverse climatic conditions curtailed the work considerably, and rendered it most improbable that any significant data could be expected to result from plots laid down for recovery trials.

It has not been possible to include in this report data relating to any differences which may be exhibited by the seed of different varieties in

resistance to attack by the frit fly, because the analysis of fifty thousand seeds, for determination of infestation, is a laborious process which will occupy many months.

I have great pleasure in acknowledging herewith the courtesy of Prof. Ehle, the Director of the Institute, and of each member of his staff. They all received me most cordially, provided me with every facility which the Institute could offer and generally extended to me all possible assistance, taking marked interest in this investigation. In particular, I should like to express my very grateful thanks to Dr Åkerman, who is in charge of nearly all the cereal investigations, for his most generous assistance and for placing his extensive knowledge of cereals most freely at my disposal.

II. THE POSITION OF THE FRIT FLY PROBLEM IN 1926.

The frit fly is an insect which has a wide distribution in corn-growing countries and which is of grave importance because its larvae destroy the growing points of shoots and seeds of the oat plants in spring and summer respectively. Limitations of the activity of the fly by chemical or mechanical means of present applicability is considered to be impracticable because of the low net value of the crop per unit area. Even if a practicable control measure of this nature were to be discovered, because of its palliative nature, efforts to establish effective preventive measures must still be made. Research work both here and abroad in relation to the frit fly is directed towards the discovery of a type of oat plant resistant to the attack of the larva. To attain this end it is necessary to determine (1) whether differences in susceptibility to infestation do exist between plant and plant, either of the same or different races or varieties, or between the growth stages of an individual plant, (2) what the characters are which tend to make a plant resistant or immune to attack, and (3) how such characters are or can be associated with capacity for yielding the maximum amount of grain of desirable quality.

To solve the frit fly problem we must increase our knowledge of plant resistance and combine it with our knowledge of the insect cycle. From these aspects, the outstanding facts discovered recently are as follows:

1. That the maximum prevalence periods of the fly in the field are fairly constant in time from season to season; the three generations of adult flies have appeared in maximum numbers about May 26th, July 15th and August 22nd, every season for the last six years (1,2,3).

2. That the shoot and seed have definite growth stages within which they are most susceptible to attack; the shoot is most susceptible while in the two or three leaf stages; from the four leaf stage onwards susceptibility drops quickly (7, 4); the grain is most susceptible about the time of fertilisation and the highly susceptible period closes before the grain reaches half its normal size (9).

3. That varieties of oat plants do show variation in extents of shoot and grain infestation, when grown under similar conditions (5). Of all the varieties submitted to experiment in England, Goldfinder and Supreme are, as judged by our present system of analysis, the varieties least and most susceptible to infestation, respectively. March sowings, for three or four years past, have shown consistent and significant differences in extent of shoot infestation of the order of 20 per cent. in favour of Goldfinder. Unfortunately, seed infestation is not correlated with shoot infestation. Later sowings in April, designed to throw the plants into the fly period about May 26th, while they were in the early leaf stages, showed that under such conditions there were no significant differences in extent of infestation, *i.e.* no appreciable differences in resistance. The difference in behaviour observed with the earlier sowings was evidently due to some characters which varied during growth but which at present are unknown.

As far as the spring attack is concerned efforts to solve the problem may be based on the study of either or both of the following characters, namely (1) the greatly increased power of resistance of the plant after it reaches the five-leaf stage, or (2) the power of resistance to infestation exhibited by one variety more than another. The problem would be solved, at least as far as the spring attack is concerned, if the correlation between the maximum fly prevalence period and the plant susceptibility period could be prevented. Total immunity might not always be obtained, because tillering capacity might influence extent of infestation; but we should get a shoot resistance of practical importance, particularly with the grain-producing varieties, by arranging that only the resistant stage of the important primary shoot should be subject to infestation (4). This might be accomplished in either of two ways, namely (1) by allowing the plant to have the longest growing period possible before May 26th and therefore time to produce its resistant stage before the fly becomes prevalent, which may be accomplished by early sowing, or (2) by introducing into the more susceptible heavy yielding types the characters which cause certain varieties to exhibit power of resistance, so that such characters may be effective during the earlier stages of growth.

At present the provision of a sufficiently long growing period is only practicable with spring oats by eliminating sowings after the end of February. Reckoning a period of three weeks for germination and periods of seven days for the production of each susceptible growth stage, these being five in number, the total period required to bring the plant into the five-leaf stage is 8 weeks (4). Adverse climatic conditions, such as abnormally low temperatures during March and April, may easily cause a set-back in plant growth prolonging the duration of one or more of the leaf stages. This necessitates a safety margin, probably of 2 weeks, which brings the total period, from sowing date to date of production of the resistant stage, up to 10 weeks. Thus, calculating from May 26th, it is obvious that the very latest sowing date for spring oats under English conditions should not be later than the middle of March and probably not later than the end of February for there to be any certainty of normal yield. This agrees with practical experience (10). Even then, the possibility of grain infestation has still to be faced, because of the frit fly population carried by the wild grasses, and this of course applies equally to winter sown oats. Provided, however, that the panicles are exserted early, the risk of heavy grain infestation is not great, because the majority of the second swarm of flies appear about the middle of July, at which time the grain should be halfway to maturity, and therefore reaching a resistant stage.

As circumstances often prevent early sowing, we are driven to attempt to solve the problem by the second method, *i.e.* by studying host resistance, hoping thereby to be able eventually to breed a type of plant with resistant powers in its early growth period and capable of giving high yield of quality grain.

III. RESISTANCE TO ATTACK AND ITS IMPLICATIONS.

It is necessary to understand exactly the meaning attached to the term "resistance." The relation between the factor "resistance to attack" and the measure "yield" also requires consideration, because of the entry of the unproductive shoot into the problem. Efforts at improving a variety aim at producing a type which will bear the maximum weight of best quality seed per unit area. The utility factors, quality of seed and weight of seed per panicle are independent of the number of panicles produced per unit area. The number of panicles produced depends on both the number of plants produced and the number of shoots produced per plant, that is, on inherent characters of the variety causing certain reactions to environmental conditions, spatial conditions being of

paramount importance in this respect. Therefore, in relation to infestation and consequent loss of shoots, the important factor is the number of shoots produced per unit area. A resistant variety must produce the maximum number of shoots, while a utility variety must produce the maximum weight of quality grain per unit area. Thus, from the point of view of resistance, the value of a shoot depends merely on its presence in an uninfested condition, not on its productive capacity at all. Total unproductiveness of a shoot may be regarded, in this connection, as an extreme case of variation in weight of grain produced per panicle. Therefore the quality of resistance must be considered entirely apart from yield. It is, however, of great importance to remember that resistance factors and utility factors may be combined by breeding, and further, that the utility value of the new product must always be judged on the evidence afforded by adequate yield trials in conjunction with observations on seed quality.

We have then to consider what is implied exactly by the term "resistance to attack" and how resistance ought to be measured. It would appear that resistance to attack may be either direct or indirect. In the case of the frit fly, direct resistance means that the shoot can resist larval entry directly or bring about the death of the larva before it can cause material injury. If the possibility of entry is dependent on the presence of such factors then direct resistance may be present or absent only. If, however, it depends on fluctuating characters associated with growth such as cuticular thickness, presence of silica, hairiness of surface, quality of sap, etc., then this type of resistance may appear and vary with age. It has indeed been proved that susceptibility decreases with age of shoot in the case of Abundance oats. The members of a shoot population, apparently uniform, may, in fact, not be so with regard to direct resistance, if the resistance exhibited by the members of the population depends on both these types of factors.

Indirect resistance includes what may be termed the biological factors. A greater capability of producing plants and shoots per unit area, early tillering capacity (thereby providing susceptible shoots for the larvae and allowing the primary shoots to escape), capacity for rapid growth (enabling the shoot to remove its susceptible regions from the path of the larva), and an efficient recovery power are examples of factors which may be included under this heading. Indirect resistance therefore can be expressed only as a comparative measure of inherent capabilities, as it is dependent on environmental conditions and will vary directly with them.

When considering how resistance may be measured, it will be found that a number of variables have to be considered. The number of larvae present may be equally or unequally distributed. If they are unequally distributed in the first place over so small an area as an experimental plot, obviously experimentation will be difficult there. Measurement of distribution can be made by using the same variety in plots over a large area and determining therefrom the distribution of infested shoots. In the opposite case of equal distribution, three other variables present themselves:

A. The larvae present may be

(*a*) Limited in number over the experimental area, *i.e.* less than the numbers of shoots open to attack.

(*b*) Unlimited in number over the experimental area, *i.e.* equal to or more than the numbers of shoots open to attack.

B. The shoots presented for attack by any two varieties may be

(*c*) Equal in numbers per unit area.

(*d*) Unequal in numbers per unit area.

C. The capability of resistance to attack may vary with variety, from

(*e*) No direct resistance and no indirect resistance.

(*f*) Direct resistance only present.

(*g*) Indirect resistance only present.

(*h*) Direct and indirect resistance present.

(*i*) Complete direct resistance, or immunity.

We may now compare the action of two or more varieties under these various possibilities. If resistance is completely absent, infestation will vary from certain equal infestations when the numbers of larvae are limited and the numbers of shoots equal or unequal to complete infestations when the numbers of larvae are unlimited. On the other hand, if resistance is complete, then infestation will be nil. If the larvae are limited in number and the shoots equal in number per unit area, resistance cannot be measured unless the numbers of larvae present and the numbers of shoots open to attack are at least equal, and the same applies to cases in which the shoots present are unequal in number also. If, however, the larvae are unlimited in numbers, resistance can be measured whether the shoots are equal or unequal in numbers.

Direct resistance must be measured by observation of the infestations of uniform populations of shoots, and direct comparisons may then be made. The most uniform populations under natural conditions are

provided by the primary shoots. It is now known that age of shoot is correlated with susceptibility to attack, therefore it is necessary to have some indication of the degree of variation in rates of growth of the primary shoots in the different varieties in order that we may experiment with uniform material when attempting to measure variation in direct resistance. Careful observation has established the fact that, under similar conditions of growth, there are no important differences in the rates of growth of the primary shoots (4). It must be remembered that apparent uniformity in morphological condition does not necessarily imply uniformity in physiological condition. However, some degree of morphological uniformity is as close to the ideal condition as we can get at present, and the use of such apparently uniform material very much simplifies the position. At the same time such a population approximates very closely to that grown under normal field conditions, because in the field it seems probable that the primary shoot is of paramount importance, firstly, because under commercial conditions about 70 to 90 % of the plants of the grain-bearing oat type produce only one shoot and one panicle and, secondly, because the recovery power of the plant, after the growth of its first shoot has been checked, seems to be very variable (4).

Measurements of the resistance of the total number of shoots carried per unit area are necessarily measurements of indirect plus direct resistance, and therefore are comparative measurements applicable to particular conditions only. If the flies are limited in number direct resistance can be demonstrated, but not measured, by observation of differences in infestations, because experiments will give different results according to the numbers of larvae present each year. If the flies are unlimited and the biological factors approximately constant, the observed percentage differences should be of the same order year by year. In the experiments recorded in previous publications (5, 6), therefore, indirect plus direct resistance has been measured, because all shoots have been included in the analyses and the regularity of the results allows us to assume that the larvae are always unlimited in number (within the meaning of "unlimited" stated above). It might be advisable, where possible, to use a highly susceptible variety or stage of plant as a control to demonstrate the existence of an "unlimited" number of larvae. The utility value of any variety will depend therefore on its power of resistance, which may be indirect and/or direct, and on its quality. Yield comparisons alone confuse the issue, wrongly giving an impression of progress and thereby delaying the analysis of the problem of resistance to attack.

IV. THE POSITION IN SWEDEN.

It was naturally expected that the fundamental aspects of the problem of resistance would be in receipt of attention, in view of the claims made by the Swedish investigators, and that they would be in a position to indicate lines of research relating to resistance likely to increase our knowledge of this subject. This expectation was justifiable without intimate knowledge of their conditions of working, but once this knowledge was attained, it became obviously unreasonable to expect assistance along these lines. The Institute derives support largely from the farming element and the associated commercial company, in addition receiving very limited State aid. The primary aim of each investigator is therefore the production and maintenance of increments in yields by the breeding of suitable new varieties, irrespective of the factors concerned in the improvements and, with the limitations of personnel imposed on the Institute by inadequate financial conditions, it is unlikely that they will progress beyond empirical experimentation in the near future. That they have perforce largely to suppress natural inclinations to explore the more distant problems certainly does not imply that the work of this Institute has not markedly influenced the economic aspect of crop production, but merely explains why such limitations are imposed, their presence and necessity not being obvious to the casual visitor whose impressions are created by 40 acres of plot experimentation. A typical example of the Swedish method of judgment of resistance may with advantage be quoted here. During the year 1918 various lines of a cross between Golden Rain and Black Bell II were compared with Black Bell III both for yield and resistance to frit fly attack. The figures quoted in Table I are the results of averaging the data obtained from triplicate plots. The estimation of the extent of the damage was based on visual observation of the number of panicles present, the figures 5–0 representing grading from normal to worthless stands, the departure from the normal being attributed to the frit fly, because the results of its activity had been observed on the plots earlier in the season. It is obvious that the figures quoted only measure the observer's ability to differentiate between variations in stand which may be the effect of indirect resistance only and that the measurements of yield include the effects of variations in resistance, both direct and indirect, presuming the frit fly attacks to have been unequal in the first place.

It is only fair to state that in other cases the extents of the frit fly attacks have been graded as above early in the season and that the

results of such grading have more or less correlated with the yield figures, as might reasonably be expected.

Table I.

Swedish variety trial, 1918.

Variety	Mean yield	Average estimation of damage
Golden Rain × Bell II (F_5)	0·520	3·7
Another line of same	0·542	4·2
,, ,, ,,	0·590	3·8
,, ,, ,,	0·395	3·0
,, ,, ,,	0·230	1·8
Golden Rain	0·190	1·7
Bell III	0·450	4·0
Golden Rain × Bell II (F_5)	0·220	2·3
Another line of same	0·205	2·7
,, ,, ,,	0·420	3·3
,, ,, ,,	0·425	3·7
,, ,, ,,	0·360	2·5
,, ,, ,,	0·160	2·2
,, ,, ,,	0·525	4·3
,, ,, ,,	0·600	3·5
Golden Rain	0·160	1·3
Bell III	0·510	3·8
Golden Rain × Bell II (F_5)	0·430	3·2
Another line of same	0·375	2·5
,, ,, ,,	0·340	2·0
,, ,, ,,	0·210	1·8
,, ,, ,,	0·760	4·3

From long observation of oat varieties produced by themselves or obtained from other parts of the world, they rightly deduce that constantly high yielding varieties are resistant under their conditions, and it may well be that the inferences drawn from such data would prove on investigation to be valid under other environmental conditions, but it is essential to realise that it is not justifiable to draw general conclusions from such data, because the important factor may be indirect resistance, which itself may vary under varying conditions of fly population and plant growth, such as will occur in other seasons or in other countries. Direct resistance must be proved to be present or the total resistance of each of such varieties must be determined in any new environment, before new varieties can be accepted with confidence for utilisation under other environmental conditions.

The problem of resistance having been studied at the Institute at the best very indirectly, considerable adjustment of the projected lines of

investigation was necessary. Instead of being in a position to study the more distant problems, it was necessary to establish the existence of variation in power of resistance between varieties, under Swedish conditions of growth and fly population. The first step was to make acquaintance with the biology of *O. frit* as known in Sweden. At Svalöv the results of the activity of the fly were of course well known, but accurate and detailed biological data were lacking. The Entomological Institute (Centralanstatens Entom. Avdelning) at Stockholm was visited at the end of April, as it was understood that Mr Lindblad was engaged in research on this problem, under the direction of Prof. Tullgren. Here it was found that sweepings had been made in the field during the year 1926, but that the material obtained was still awaiting examination; also that numerous parasites had been bred from the immature stages, these parasites being undetermined. It would have been very helpful if their researches into the biology of this pest had been prosecuted further, but other interests claimed their attention.

V. OUTLINE OF PROJECTED RESEARCH WORK IN SWEDEN.

The following scheme of work was outlined after the problem had been discussed with Dr Åkerman and the possible scope under the particular conditions determined:

(*a*) Data as to fly prevalence were to be collected by sweeping in the field.

(*b*) Early and late sowings of oat varieties were to be made, utilising available stocks of markedly different varieties, for the purpose of determining variations in power of resistance of primary shoots, total shoots and seed, to frit fly attack.

(*c*) The later sown plots were to be laid down with the intention of obtaining some preliminary information as to recovery power as determined by yield.

(*d*) Large comparative trials were to be laid down for confirmation of the results obtained from (*c*).

(*e*) Records of rates of growth were to be made.

(*f*) Crosses were to be made between varieties showing marked resistance to infestation and varieties having desirable yield qualities.

(*g*) Observations were to be made on other material laid down for the general purposes of the Institute work, where it was considered they might provide supplementary data.

VI. THE PREVALENCE OF THE FRIT FLY IN SKÅNE, S. SWEDEN.

Sweeping was conducted with a conical net, 12 in. in diameter, having a terminal tubular tin to facilitate the killing and transference of material collected. Fifty semicircular sweeps, as far as possible of equal value,

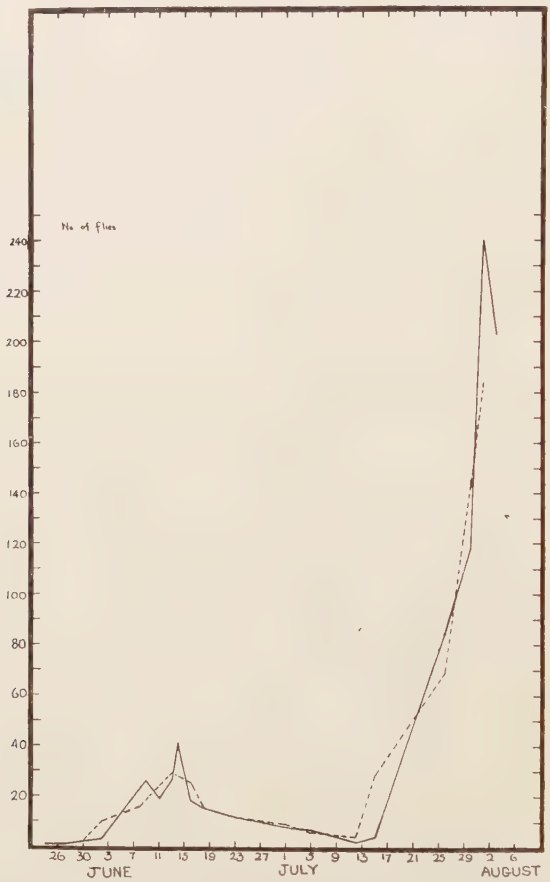

Chart I. Prevalence curve; unbroken line, from actual observations; broken line, smoothed curve.

were made over the tops of the oat plants, the same line in the field being kept on each occasion. As far as weather conditions permitted, collections were made on alternate days and the numbers of flies recorded. These data are set out in Chart I in the form of a smoothed curve, the adopted mean for any one date being the mean of the observation

recorded for that date and the two observations immediately adjacent. Smoothed curves for rainfall and maximum and minimum shade temperatures are shown on Chart II for record.

Previous to May 23rd no flies were swept from any cereal or grass crops, either in the neighbourhood of Svalöv or elsewhere. The latter part

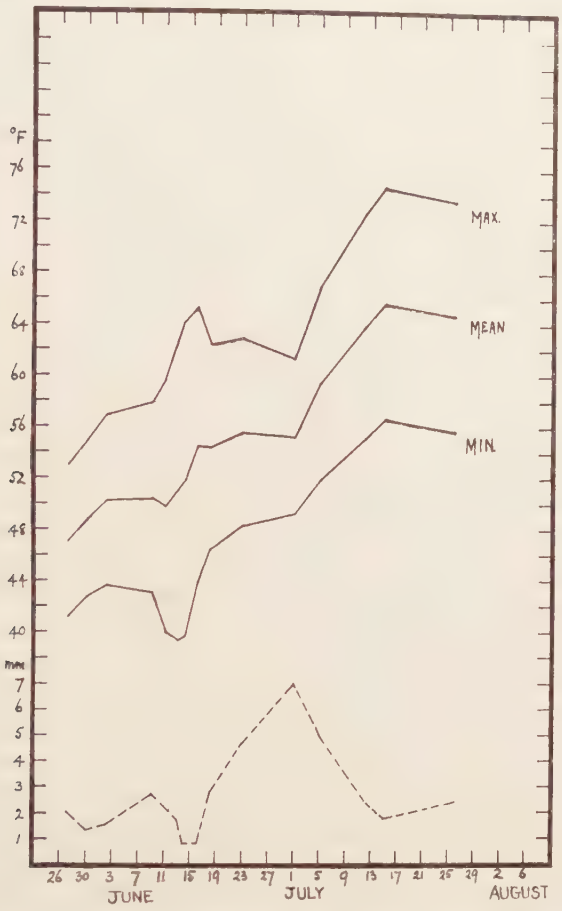

Chart II. Smoothed curves, representing, from below upward, rainfall in mm., minimum, mean and maximum shade temperatures.

of May was decidedly wet, but warm dry intervals were sufficiently numerous to show that emergence did not occur prior to May 23rd at Svalöv. The month of June was very favourable for sweeping, until about the 20th, when continuous rains and high winds prevented observations by this method. The flies were however observed at work on the

experimental plots during this wet period. Very few flies were present in the field during the first half of July and after the middle of the month frequent thunder showers prevented sweeping operations until the 26th, about which time the second swarm of the year began to emerge. The primary object of this operation, namely, the determination of the prevalence of the first swarm of the year, had been attained, but it is to be regretted that it was not possible to complete the observations at this later period. It would appear, from these data, that the duration of the period between the two maximum prevalence periods would have been about 50 days, as it is in England. The mean shade temperature over this period was 60·0° F.

VII. EXPERIMENTATION IN RELATION TO THE PROBLEM OF RESISTANCE.

(*a*) Experimental procedure and data.

The vagueness of the data relating to the biology of the frit fly made it difficult to prearrange the sowing periods, so in order that it should be certain that the susceptible stages of plant growth should be produced while the flies were in active oviposition, the first sowing was made at hazard on April 25th to 28th, and the second when the first fly was caught in the field, namely, on May 23rd.

Thirty-two varieties of oats, markedly different from one another in various characters such as yielding capacity, colour, tillering capacity and country of origin, etc., were suggested for experiment by Dr Åkerman, who has been making annual visual examinations of scores of different types for many years; these varieties he selected as likely, from such experience, to be productive of result, direct or comparative. At the same time, limitation of experimentation was imposed by two conditions, namely (1) that the supply of labour, both for field work and analysis, was strictly limited, and (2) that it was considered desirable to utilise only such varieties as would be likely to produce, on crossing, types of value to the English agriculturist. The following thirty-two varieties were used in these sowings, namely: King (01171 b); Star (01182); Victory (0355); Golden Rain (0386); Golden Rain II (01221 c); Ligowo (0353), originally from Russia; a cross between Lochows Yellow and Victory (01272); Lochows Yellow, a German yellow oat; Lüneburger Kley, common north of Hamburg; Leutewitzer, an old German yellow oat; Weibull's Echo and Weibull's Early, both lines from a cross between Golden Rain and Leutewitzer; White Yeoman (01164 c); selected Dala (0924), a country variety from middle Sweden; Gophers, from U.S.A.;

White or Kytö, from Tammisto, in Finland; Spet, a country variety from Småland, Sweden; Hede, from Denmark; Summer, from Gotland, in the Baltic; Black Bell II (0408); Engelbrekt (01150 e); Great Mogul (0450); Weibull's Argus; Roslags, a country variety from an island east of Stockholm; Plume or Black Tartar (0210); Black Supreme; Orion II (01104); Mesdag, a Finnish country oat, selected in Holland; Sandy; Tam Finlay; Kent Berlie; and finally *Avena fatua*[1].

The seed was sown by means of the sowing board in the normal manner, at a spacing of 12·5 × 5 cm. and a depth of 5 cm. on plots carrying four rows, each of 30 seeds, for each variety. The order of sowing was that indicated in the above list of varieties, except that additional plots of Victory oats were inserted to act as controls, the location of this variety being in the plots numbering 3, 13 and 26 respectively. The earlier sowing (April 25th) was replicated twice and the later sowing (May 23rd) five times, the unequal division being due to the limited supply of labour available, to the fact that it was obviously more advantageous to concentrate on the later sown plots, which were certain to be attacked, and finally to the fact that it was desired to obtain from these plots some information as to the recovery power of the different varieties, for which purpose it was essential to have at least six observations of yield for analytical purposes. By May 12th the majority of the first sown plots were showing through, Black Supreme alone failing to appear by this date[2]. The latter part of May, as may be seen by reference to Chart II, was very cold and wet and these conditions were reflected in the poor germination of the seed in this first series. From 120 seeds, the minimum, mean and maximum numbers of plants obtained per plot were 39, 79 and 110 respectively. The plants from the later sowing commenced to break through on June 1st, being produced much more evenly than those of the first sowing, owing to the better climatic conditions prevailing. In this case, from 120 seeds, the minimum, mean and maximum numbers of plants obtained per plot were 32, 102 and 118 respectively. The rate of growth of the primary shoots was determined by observing the date of appearance of the leaves in order, in particular of certain varieties considered by Dr Åkerman to be early in habit. Typical observations made on these particular varieties are shown in Table II.

Late germinations were responsible for the deviations of Golden Rain, Summer, Orion, and Mesdag on May 25th. The early start of such varieties as Lochows Yellow, Gophers, Summer and Mesdag oats was

[1] A number following the name of a variety indicates the Svalöv identification number.

[2] This seed was old and therefore many were not viable.

Table II.

Showing percentages of plants in different leaf stages at different dates.

Variety	Percentage of plants in second-leaf stage			Percentage of plants in third leaf	Percentage of plants in fourth leaf
	May 18	May 21	May 25	May 30	June 4
Victory	0	59	100	79	87
Golden Rain	0	63	94	61	84
Golden Rain II	16	96	100	79	92
Lochows Yellow × Victory	21	71	100	93	93
Lochows Yellow	55	91	100	96	88
Weibull's Early	21	93	100	97	83
Gophers	48	87	100	91	78
Spet	33	89	100	89	90
Summer	57	70	96	96	96
Engelbrekt	14	71	100	91	75
Orion	14	94	94	88	82
Mesdag	87	87	85	79	87
Tam Finlay	14	76	100	95	97
Avena fatua	0	20	100	60	39

not maintained, no differences in rates of growth of practical importance being exhibited by the varieties; *Avena fatua* was exceptional, but even in this case, the lag was comparatively slight.

With the later sowings, the plants were in the second-leaf stage by June 7th, in the third-leaf stage by June 14th and in the fourth-leaf stage by June 18th, the rate of leaf production being very even, varieties such as Mesdag being no more advanced than any others. Other observers from members of the staff confirmed the absence of material variation in the rate of growth of the primary shoot and, as all these observations confirmed detailed observations of the same type made in England during the years 1925 and 1926 (4) it was considered unnecessary to amplify them.

The primary shoots of the plants in the earlier sown plots passed from the first- to the fourth-leaf stage during the period May 18th to June 4th. Reference to Chart I will show that these shoots were hardly exposed to the attack of the fly before they reached the stage of comparative immunity. Their tillers, however, were continually exposed to attack. In the case of the later sown plants, the primary shoots were in their most susceptible stage, namely, the early three-leaf stage, on June 14th, at which period the prevalence of the flies was at its maximum.

On June 11th the primary shoots of the plants of the first sowing were marked with red wool for later identification, as they were then in the fifth- and early sixth-leaf stages. The later sown plants were only in the

late two-leaf on this date and as it was considered that their identification would not be difficult when it came to analysis they were left unmarked.

The analysis of the shoots for estimation of extent of attack was commenced on July 1st, that is, about 17 days after the period of maximum prevalence of the earliest swarm of flies, by which time the results of oviposition should theoretically have been apparent visually, but to avoid possibility of error in this direction each shoot was split and when necessary examined under the binocular microscope. Samples, consisting of the products of one drill, were drawn from each plot of each variety and

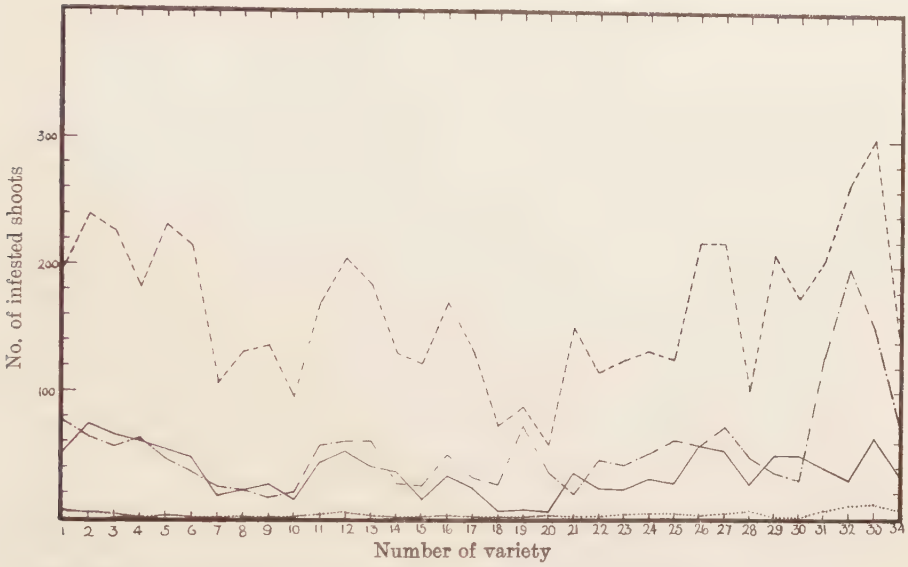

Chart III. Order of infestation shown by each variety; first sowing, three series and second sowing, six series; dotted line = first sowing, primary shoots; broken dotted line = first sowing, total shoots; unbroken line, second sowing, primary shoots; broken line, second sowing, total shoots.

examined in regular sequence, in order to overcome the error which would otherwise have been introduced by late hatching in conjunction with delayed examination. All the varieties composing one series were sampled at the same time.

The necessity of ordered sampling and analysis becomes obvious when it is considered that this analysis alone involved the individual examination of 36,800 shoots. The minimum, mean and maximum numbers of shoots examined in each series were as follows: *first sowing*, primary shoots, 19, 55 and 78; total shoots 102, 315 and 683: *second sowing*, primary shoots,

79, 152 and 178, total shoots, 242, 561 and 813, respectively. It should be noted that the plot replications numbered two for the first sowing, but five for the second sowing. Records were kept of the numbers of unattacked and attacked primary shoots and also the numbers of unattacked and attacked visible or total shoots per plant. The order of the infestation for each series is shown graphically in Chart III, in the form of curves, the actual numbers of infested shoots observed for each variety being indicated. The order of the plots[1] in the field may be indicated as follows:

II 21	II 22	V 25	V 26	VIII 29	VIII 30
↑	↓	↑	↓	↑	↓
I 1	IV 5	IV 6	VII 9	VII 10	IX 36

In Table III are shown the percentage infestations of the shoots for the varieties of oats under observation, in the order of sowing, together with the standard errors of same. The percentage infestation has been derived from the summation of the data obtained from all the plots of the same variety grown under the same conditions, on the assumption that the fly population was uniformly distributed and therefore that each plot could be treated, legitimately, as a sample from a large area. The size of the shoot population has been brought into the determination of the standard error by the utilisation of the formula $e = \sqrt{\frac{P(100-P)}{n}}$ where $P =$ the percentage infestation and $n =$ the size of the shoot population. A second analytical method is indicated in Section IX.

(*b*) Discussion of data.

The plots of Victory oats numbered twenty-seven and as they were distributed over the whole area, it is possible to determine approximately the type of distribution of the attack and, therefore, of the fly, over the area. The actual numbers of infested primary and total shoots are shown in Chart IV for each plot together with smoothed curves indicating the results of averaging the members of the series in groups of five.

The actual numbers of infested shoots were, as usual, very variable in all the series. How far this was due to inadequate sampling, the irregularity of oviposition or to variation in plant characters, it is impossible to determine in this case, but the necessity for statistical examination of any results, deduced from data of this type, is very obvious. The

[1] Actually there were thirty-six plots in each series, because the field plots numbering 31 and 32 were put down to very early maturing barleys, namely, Six-row barley from Norrbotten (N. Baltic region) and Golden Barley, for the information of Dr Åkerman. Neither of these barleys suffered infestation of practical importance, only a few odd stems containing larvae.

Table III.

Percentage infestation with standard error.

Variety in order of sowing	First sowing		Second sowing	
	Primary shoots	Total shoots	Primary shoots	Total shoots
1. King	12·2 ±5·1	30·2 ±2·9	31·1 ±3·6	33·8 ±2·0
2. Star	7·3 ±3·5	21·2 ±2·3	43·2 ±3·8	41·5 ±2·0
3. Victory	5·6 ±3·1	17·9 ±2·2	38·2 ±3·7	36·2 ±1·9
4. Golden Rain	0	22·2 ±2·5	34·4 ±3·6	32·7 ±2·0
5. Golden Rain II	2·8 ±1·9	13·4 ±1·8	32·6 ±3·6	36·6 ±1·9
6. Ligowo	1·8 ±1·8	14·4 ±2·2	28·1 ±3·4	36·3 ±2·0
7. Lochows × Victory	0	6·3 ±1·2	11·8 ±2·5	19·3 ±1·7
8. Lochows	1·5 ±1·5	6·6 ±1·4	14·0 ±2·8	24·7 ±1·9
9. Lüneburger Kley	0	5·0 ±1·2	17·2 ±3·0	24·4 ±1·8
10. Leutewitzer	2·7 ±2·7	8·9 ±1·9	12·5 ±3·0	20·8 ±1·9
11. Echo	4·7 ±2·6	16·5 ±2·0	31·2 ±3·8	35·4 ±2·2
12. Early	6·7 ±2·9	15·8 ±1·9	30·6 ±3·5	33·3 ±1·9
13. Victory	3·3 ±2·3	18·6 ±2·1	23·6 ±3·2	30·0 ±1·8
14. White Yeoman	0	7·1 ±1·7	21·2 ±3·1	24·4 ±1·9
15. Dala	2·4 ±2·4	6·7 ±1·6	11·2 ±2·7	23·7 ±1·9
16. Gophers	3·2 ±2·2	13·4 ±1·7	19·9 ±3·0	25·5 ±1·7
17. Kytö	1·5 ±1·5	10·1 ±1·7	14·3 ±2·7	25·2 ±1·9
18. Spet	1·8 ±1·8	7·8 ±1·5	5·0 ±2·0	13·4 ±1·5
19. Hede	1·4 ±1·4	13·1 ±1·4	5·2 ±1·9	12·3 ±1·2
20. Summer	4·0 ±2·8	11·8 ±1·8	3·8 ±1·7	11·2 ±1·4
21. Black Bell II	5·3 ±5·1	18·6 ±3·8	22·3 ±3·3	30·2 ±2·0
22. Engelbrekt	1·5 ±1·5	14·0 ±1·9	14·1 ±2·7	20·8 ±1·7
23. Great Mogul	8·3 ±4·6	21·2 ±2·9	13·8 ±2·7	22·2 ±1·7
24. Argus	8·3 ±4·0	23·2 ±2·8	21·2 ±3·4	32·4 ±2·3
25. Roslags	5·2 ±2·5	14·4 ±1·7	15·7 ±2·7	21·6 ±1·7
26. Victory	3·4 ±2·4	19·0 ±2·2	33·0 ±3·5	35·2 ±1·9
27. Black Tartar	5·8 ±2·8	21·4 ±2·2	38·3 ±4·1	40·3 ±2·1
28. Black Supreme	20·0 ±7·3	27·1 ±3·3	33·8 ±5·3	41·8 ±3·2
29. Orion	2·1 ±2·1	17·1 ±2·6	29·4 ±3·5	34·8 ±1·9
30. Mesdag	0	13·9 ±2·3	36·8 ±4·1	40·4 ±2·4
31. Sandy	12·2 ±4·7	33·8 ±2·4	33·9 ±6·2	34·2 ±1·9
32. Tam Finlay	15·2 ±4·4	29·1 ±1·7	21·2 ±4·8	32·6 ±1·6
33. Kent Berlie	20·3 ±5·5	36·6 ±2·4	41·9 ±5·7	42·5 ±1·8
34. *Avena fatua*	21·8 ±7·3	29·9 ±2·9	44·3 ±5·6	37·0 ±2·3
35. Victory (plots 3, 13 and 26)	4·1 ±1·5	18·5 ±1·3	31·5 ±2·0	33·7 ±1·1

smoothed curves rise quickly in the cases of the total shoots and very slightly in the case of the primary shoots of the second sowing. This may mean that the attack was more concentrated on certain parts of the area or that the measurable attack increased with delayed examination. Other varieties occupying intermediate positions, such as Kent Berlie and

Golden Rain II, suffered equally as severely as Victory, tending to show that the attack was not particularly concentrated. The comparative regularity of the mean curve for the primary shoots of the later sown plants, which reached the fourth-leaf stage about the middle of June and therefore would be practically immune by the middle of July, proves that the indicated rise in infestation was due to delayed sampling. The analyses were necessarily made single-handed and occupied a period of about three weeks. It is of importance to know to what degree the flies are limited in numbers in the field because, when studying differences in resistance,

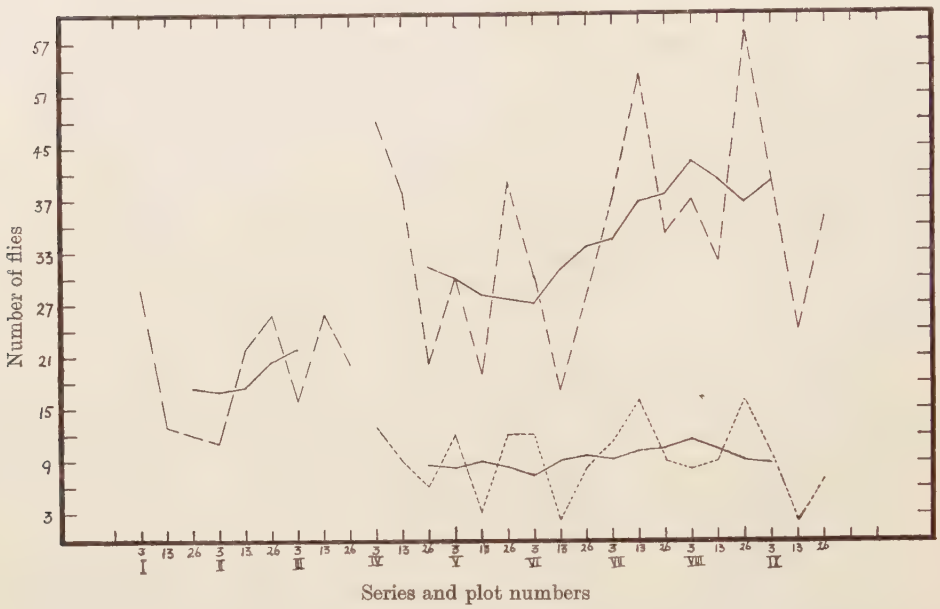

Chart IV. Distribution of infestation, as shown by plots of Victory oats. Dotted line, second sowing, primary shoots; broken lines, first and second sowings, total shoots; unbroken lines, mean curves.

it is essential to know that all susceptible shoots have been exposed to attack. If the number of shoots (T) is constant, then the percentage infestation $P = \frac{I}{T} \times 100$ will increase directly with the actual infestation (I). If the number of flies in the field is limited, then I will become constant for the highly susceptible varieties when their susceptible shoots number more than the larval supply and P will also become constant, unless T is also variable. In plot experiments of this type T is likely to vary but little from plot to plot for most of the varietal types used for

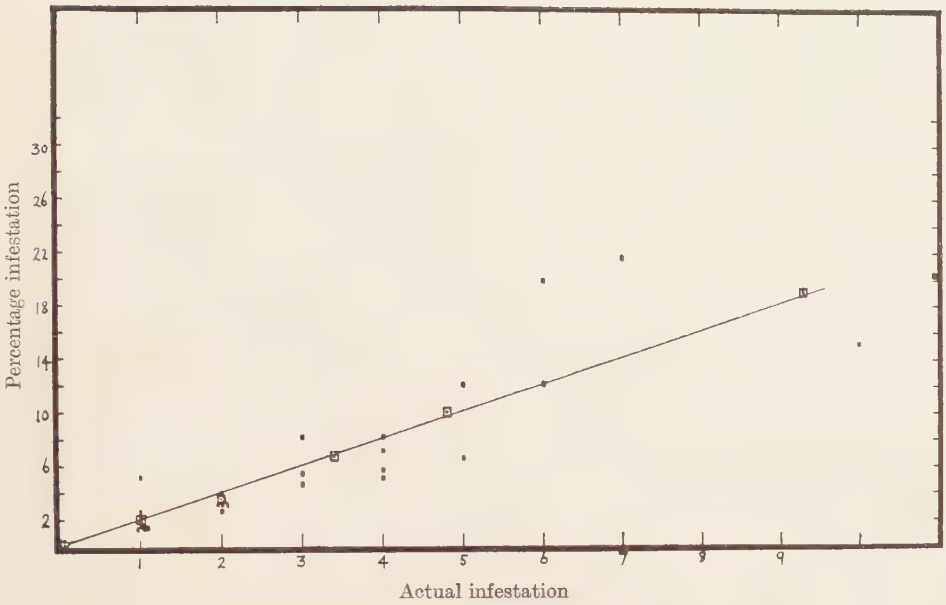

Chart V. First sowing, primary shoots.

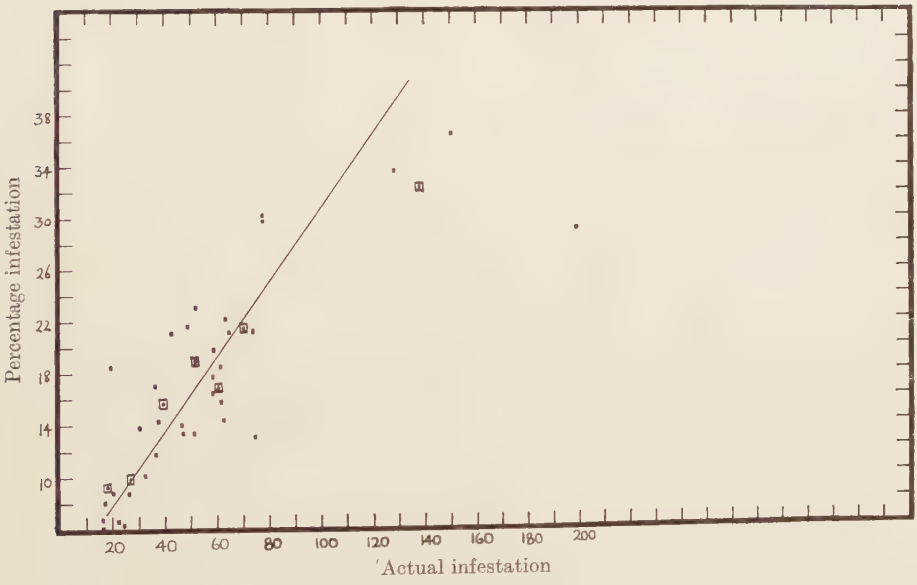

Chart VI. First sowing, total shoots.

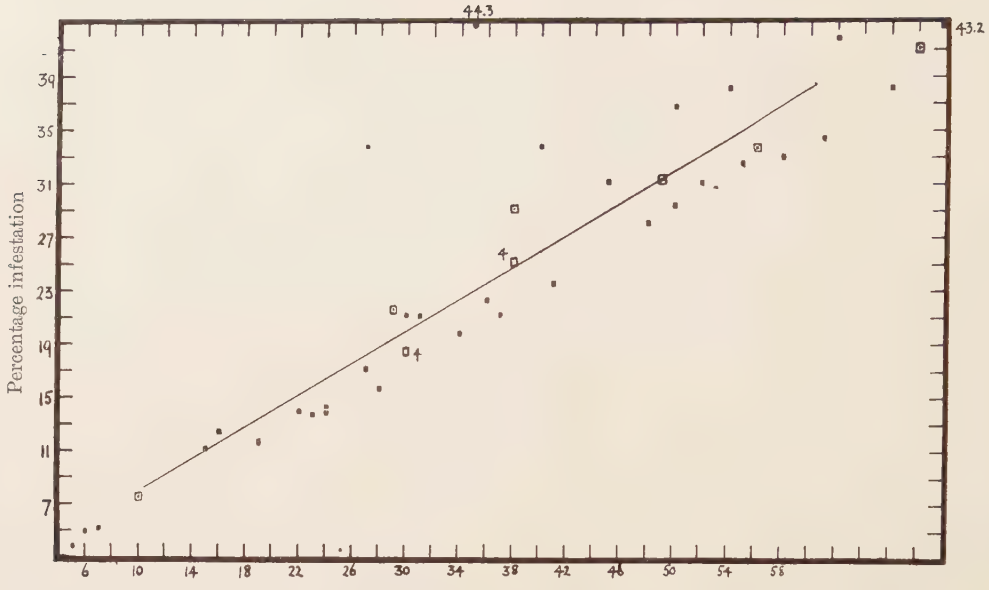

Chart VII. Second sowing, primary shoots.

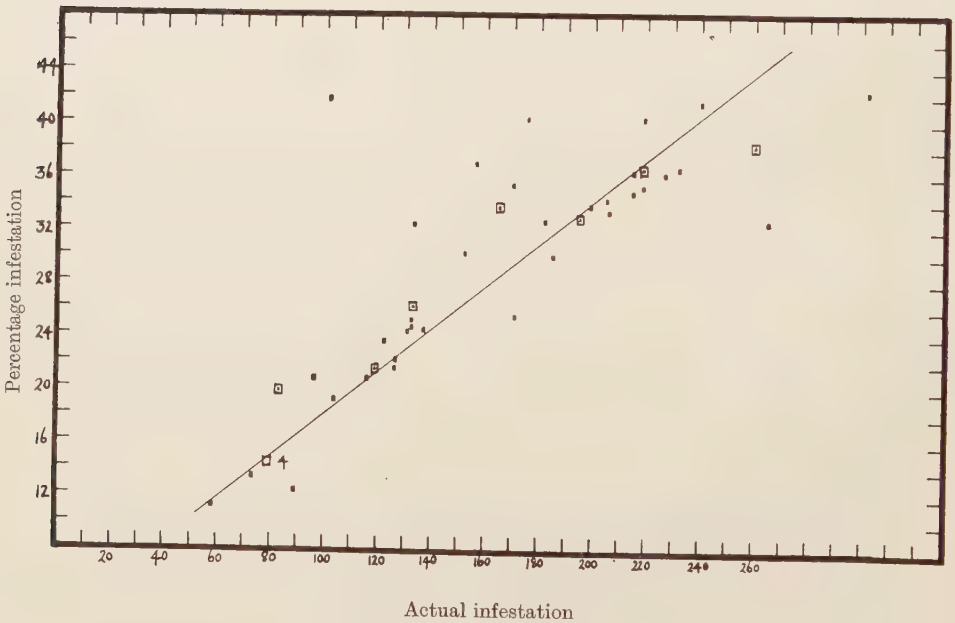

Chart VIII. Second sowing, total shoots.

experimentation, and any indication of the constancy of I will probably imply a limitation of the fly population. If T varies, then the curve of P will tend to depart from the straight line and become parallel to one of the axes. In Charts V to VIII the percentage infestations have been plotted against the actual infestations for each series and the mean curves plotted by taking the means of the observations in sets of five. It will be noted that the mean curves are in the form of straight lines, showing that for the majority of the varieties sufficient numbers of larvae to attack the susceptible shoots were present. If the larvae were limited in numbers, then the mean curve should tend to become parallel to the percentage axes, I then being a constant and T variable. None of the mean curves show any very obvious departure from the straight line and therefore it would seem justifiable to assume that the larvae were unlimited, *i.e.* present in sufficient numbers to attack all the susceptible shoots present in the majority of cases. Isolated records well below the mean curve signify that for that particular case the larvae were limited in numbers and therefore that the true susceptibility was not being measured. A case in point is shown on Chart VI, where Tam Finlay, with an actual infestation of 199, shows a percentage infestation of only 29·1. What actually occurs is that tillering is rapid when the prevalence of the fly is on the wane. A record well above the mean curve implies that the stand of shoots was much below the normal and probably also that their growth was deficient, therefore their susceptibility high. These facts may be expressed numerically by the coefficient of correlation between percentage infestation and actual infestation, as shown in Table IV. These indices also show that resistance may be measured either by the percentage or the actual infestation, when the larvae are unlimited in numbers, and suggest that the importance of tillering capacity still requires detailed study from this point of view (section C). The low indices obtained within the comparative series are due to the unequal occurrence of tillering without equivalent exposure to infestation.

The distribution of the plots of Victory oats was arranged to determine the variation over the area in the distribution of attacked shoots, and reference to Table III will show that the percentage infestation of shoots in the same stage of growth was approximately uniform. The primary shoots of the second sowing formed an exception, but here the coefficient of variation was extremely high (79·5) as compared with plots 3 and 26 (16·6 and 38·2 respectively). On the whole, the distribution may be considered to be approximately uniform and therefore that the data obtained from each group of the Victory plots may be considered together

Table IV.

Coefficients of Correlation between actual and percentage infestations.

A. *First sowing.*	
1. Primary shoots:	
(*a*) Whole series	+0·96 ±0·01
(*b*) Omitting Tam Finlay and Kent Berlie	+0·89 ±0·02
(*c*) Omitting Supreme, Sandy, Tam Finlay, Kent Berlie and *Avena fatua*	+0·90 ±0·02
2. Total shoots:	
(*a*) Whole series	+0·78 ±0·04
(*b*) Cases where actual numbers of shoots infested reached 80 or less	+0·72 ±0·06
(*c*) Cases where actual numbers of shoots infested reached 60 or less	+0·64 ±0·08
B. *Second sowing.*	
1. Primary shoots:	
(*a*) Whole series	+0·87 ±0·03
(*b*) Omitting Supreme and *Avena fatua*	+0·94 ±0·01
2. Total shoots:	
(*a*) Whole series	+0·74 ±0·05
(*b*) Cases where actual numbers of shoots infested reached 200 or less	+0·70 ±0·07
(*c*) Cases where actual numbers of shoots infested reached 175 or less	+0·86 ±0·04
C. *Percentage infestations of the different series.*	
1. Same series, primary and total shoots:	
(*a*) First sowing	+0·84 ±0·03
(*b*) Second sowing	+0·95 ±0·01
2. Comparative series, first and second sowings:	
(*a*) Primary shoots	+0·49 ±0·09
(*b*) Total shoots	+0·63 ±0·07

to provide standards of comparison for the other varieties. These results are shown at the bottom of Table III.

It now remains to determine the significance of the differences, which is readily determined in any one case by calculating the standard error of the difference (E) from the standard errors of the observed percentage infestations ($E = \sqrt{E_1^2 + E_2^2}$) and dividing the observed difference by this figure. In this way, a measure of significance is obtained which has great value in determining the degree of reliance which may be placed on such observed differences. When the measure of significance is of the order 2·57, then the chances are 100 to 1 in favour of the result. For this work a standard of 100 to 1 has been adopted, because it is of the utmost importance that the existence of a difference should be firmly established before varieties showing desirable characters should be utilised for breeding purposes. By such means also it should be possible to establish an order of merit, based on the evidence provided by each series. The data recorded in Table III have been subjected to statistical analysis, to

determine the significance of the observed differences, where such differences appear to be of some weight, and the results of such analyses are shown in Tables V to VIII.

Table V.

First sowing, primary shoots.

Varieties differing from Victory by more than 10 %.

Ref. no.	Variety	Difference between percentage infestations	Standard error of difference	Measure of significance
34	*Avena fatua*	+17·7	7·5	2·37
33	Kent Berlie	+16·2	5·7	2·85
28	Supreme	+15·9	7·5	2·13
32	Tam Finlay	+11·1	4·7	2·38
35	Victory*	—	—	—

* Variety with which the above have been compared (infestation 4·1 %).

First sowing, primary shoots.

Although Chart V shows that there were sufficient larvae present to attack the susceptible primary shoots, there were apparently very few susceptible shoots present, as these shoots had reached the fourth-leaf stage by the beginning of the prevalence period of the fly. The majority of the varieties differed from Victory by less than 10 per cent. and have therefore been omitted from Table V. Of this series, therefore, Kent Berlie was the only variety definitely more susceptible to attack than Victory, although Tam Finlay and *Avena fatua* nearly approach the necessary standard.

On the other hand, Golden Rain, the cross between Lochows and Victory, Lüneburger Kley, White Yeoman and Mesdag escaped attack on the primary shoots altogether, but cannot be said to be of a more desirable type than Victory, from the point of view of direct resistance, as judged by the evidence afforded by this particular series.

First sowing, total shoots.

Here the complication of tillering is introduced and direct plus some indirect resistance is really being measured. Only those varieties which differed from Victory by more than 5 per cent. have been considered in the above synopsis. In this case, when the total numbers of shoots produced on the plots were considered, Kent Berlie, Sandy, King, *Avena fatua*, Tam Finlay and Supreme proved to be definitely less resistant than Victory. Among the twelve varieties which were significantly less susceptible to attack than Victory oats, differences of the order of 5 per cent.

Table VI.

First sowing, total shoots.

Varieties differing from Victory by more than 5%.

Ref. no.	Variety	Difference between percentage infestations	Standard error of difference	Measure of significance
33	Kent Berlie	+18·1	2·7	6·72
31	Sandy	+15·3	2·7	5·58
1	King	+11·7	3·2	3·68
34	*Avena fatua*	+11·4	3·1	3·64
32	Tam Finlay	+10·6	2·1	4·93
28	Supreme	+ 8·6	3·6	2·42
36	Victory*	—	—	—
5	Golden Rain II	− 5·1	2·2	2·30
16	Gophers	− 5·1	2·2	2·37
19	Hede	− 5·4	1·9	2·83
20	Summer	− 6·7	2·2	2·98
17	Kytö	− 8·4	2·1	3·98
10	Leutewitzer	− 9·6	2·3	4·20
18	Spet	−10·7	1·9	5·54
14	White Yeoman	−11·4	2·1	5·48
15	Dala	−11·8	2·1	5·76
8	Lochows	−11·9	1·9	6·40
7	Lochows × Victory	−12·2	1·8	6·87
9	Lüneburger Kley	−13·5	1·7	7·82

* Variety with which above have been compared (infestation 18·5 %).

have significance and therefore these particular varieties may be placed in an order of merit, those showing a percentage difference in infestation below − 7 per cent. forming a group definitely less resistant than those composing the group infested to the extent of − 12 per cent. and above. But it is not possible here to distinguish between Spet and Lüneburger Kley.

Second sowing, primary shoots.

Here the populations are approximately uniform in character. Within this series, in addition to those varieties which only differ from Victory by 5 per cent. or less, it is not possible to differentiate between Victory and *Avena fatua*, Star, Kent Berlie, Black Tartar, Mesdag, Black Bell II and Tam Finlay, in spite of the extent of some of the observed differences. The remaining fifteen varieties were, however, significantly more resistant than Victory. Considering this group alone, detailed analysis indicates that differences in percentage infestation of the order of 10 per cent. and above are significant, therefore those varieties showing a difference of − 21·5 per cent. and above, namely Hede, Spet and Summer, were

Table VII.

Second sowing, primary shoots.

Ref. no.	Variety	Difference between percentage infestations	Standard error of difference	Measure of significance
34	*Avena fatua*	+12·8	5·9	2·16
2	Star	+11·7	4·2	1·59
33	Kent Berlie	+10·4	6·1	1·71
27	Black Tartar	+ 6·8	4·6	1·49
30	Mesdag	+ 5·3	4·6	1·15
35	Victory*	—	—	—
21	Black Bell II	− 9·2	3·9	2·38
14	White Yeoman	−10·3	3·7	2·78
24	Argus	−10·3	3·9	2·62
32	Tam Finlay	−10·3	5·2	1·96
16	Gophers	−11·6	3·7	3·16
9	Lüneburger Kley	−14·3	3·6	3·94
25	Roslags	−15·8	3·4	4·64
17	Kytö	−17·2	3·4	5·08
22	Engelbrekt	−17·4	3·3	5·20
8	Lochows	−17·5	3·4	5·11
23	Great Mogul	−17·7	3·3	5·28
10	Leutewitzer	−19·0	3·6	5·22
7	Lochows × Victory	−19·7	3·3	6·07
15	Dala	−20·3	3·4	6·00
19	Hede	−26·3	2·8	9·38
18	Spet	−26·5	2·8	9·32
20	Summer	−27·7	2·6	10·50

* Variety with which the others have been compared (infestation 31·5 %).

definitely more directly resistant than those showing a difference of only − 11·5 or less, namely White Yeoman, Argus, Gophers and Lüneburger Kley. The comparison of data from such population of primary shoots has greater significance than when the total shoots are considered.

Second sowing, total shoots.

Again only those varieties which differed from Victory by more than 5 per cent. have been tabulated. Kent Berlie and Supreme alone maintained their position as being less resistant than Victory. In addition, this series indicates that Star, Mesdag and Black Tartar may under certain conditions suffer more heavily than Victory. In consequence of the population of shoots included being much greater in this series than in the previous series, the errors were much reduced and examination of those varieties showing greater resistance than Victory indicates that

Table VIII.

Second sowing, total shoots.

Ref. no.	Variety	Difference between percentage infestations	Standard error of difference	Measure of significance
33	Kent Berlie	+ 8·8	2·1	4·08
28	Supreme	+ 8·1	3·4	2·42
2	Star	+ 7·8	2·3	3·37
30	Mesdag	+ 6·7	2·6	2·57
27	Black Tartar	+ 6·6	2·4	2·78
35	Victory*	—	—	—
16	Gophers	− 8·2	2·0	4·08
17	Kytö	− 8·5	2·2	3·87
8	Lochows	− 9·0	2·2	4·17
9	Lüneburger Kley	− 9·3	2·1	4·38
14	White Yeoman	− 9·3	2·1	4·32
15	Dala	− 10·0	2·2	4·63
23	Great Mogul	− 11·5	2·0	5·61
25	Roslags	− 12·1	2·0	6·02
10	Leutewitzer	− 12·9	2·2	5·88
22	Engelbrekt	− 12·9	2·0	6·32
7	Lochows × Victory	− 14·4	2·0	7·20
18	Spet	− 20·3	1·8	11·20
19	Hede	− 21·4	1·6	13·05
20	Summer	− 22·5	1·4	15·65

* Variety with which above have been compared (infestation 33·7 %).

here differences in percentage infestation of the order of 6 per cent. and above are significant. Therefore, in this series Spet, Hede and Summer proved definitely to be more resistant to attack than the remainder of the series.

(*c*) Interpretation of data.

The evidence derived from the study of the extent of the attack on the primary shoots of the later sowing indicated that Hede, Spet and Summer are varieties likely to be most suitable for breeding purposes. The varieties which appear in each of the three series (the first series of primary shoots providing no good evidence), and which therefore may be said to be more resistant than Victory, are Lochows × Victory, Lochows, Lüneburger Kley, Leutewitzer, White Yeoman, Dala, Kytö, Sept, Hede and Summer. The evaluations of differences in percentage infestation, observed to exist between these varieties and Victory, for the two series including all the shoots, are not of the same order in the case of each variety, therefore these values have been combined to obtain a weighted mean figure for each variety, for comparison with the results obtained from the primary

shoots of the later sowing. The results of these combinations are shown in Table IX.

Table IX.

Weighted mean differences in percentage infestations, total shoots.

Variety		Difference between percentage infestation, compared with Victory	Standard error of difference, squared	Weighted mean difference in percentage infestation	Standard error of weighted mean difference
Kytö	(*a*)	− 8·4	4·46	− 8·5	1·54
	(*b*)	− 8·5	4·77		
White Yeoman	(*a*)	−11·4	4·33	−10·3	1·49
	(*b*)	− 9·3	4·64		
Lochows	(*a*)	−11·9	3·45	−10·6	1·41
	(*b*)	− 9·0	4·67		
Dala	(*a*)	−11·8	4·20	−11·0	1·49
	(*b*)	−10·0	4·71		
Leutewitzer	(*a*)	− 9·6	5·21	−11·3	1·58
	(*b*)	−12·9	4·80		
Lüneburger Kley	(*a*)	−13·5	3·06	−11·8	1·35
	(*b*)	− 9·3	4·47		
Lochows × Victory	(*a*)	−12·2	3·15	−13·3	1·32
	(*b*)	−14·4	4·01		
Hede	(*a*)	− 5·4	3·61	−14·6	1·24
	(*b*)	−21·4	2·68		
Spet	(*a*)	−10·7	3·72	−15·8	1·32
	(*b*)	−20·3	3·32		
Summer	(*a*)	− 6·7	5·01	−17·6	1·20
	(*b*)	−22·5	2·07		

(*a*) = first sowing; (*b*) = second sowing.

With these weighted means a difference of 5 per cent. is significant, that is, varieties giving values of differences above 15·5 per cent. are sharply differentiated from varieties giving values below 10·5 per cent. Therefore Summer and Spet are significantly most resistant on the evidence of both series and also are most directly resistant as shown by the series comprising primary shoots alone. The varieties occupying the intermediate positions may be associated with either of the extreme types, but the character of the available data precludes any definite expression of opinion as to their comparative resistant powers, amongst themselves. All the varieties shown in Table IX are of course more resistant to attack than Victory oats.

(*d*) Other observations on varietal resistance.

Certain other observations, on a much less extensive scale, were made on some of the varieties used in the previous experiments, also on different varieties, to determine firstly whether different conditions would exert

much influence on differences in resistance, and secondly, whether any yield data obtained from the previous experiments would be confirmed by experimentation on a somewhat larger scale. These data are recorded in the following sections, under the headings (1) large comparative trial, and (2) data from observation plots. In a third section a Lochows × Victory crossing, actually bred for commercial purposes, is compared with Victory and Lochows from the standpoint of resistance.

1. *Large comparative trial.*

Certain of the varieties, namely Victory, Golden Rain, Black Bell II, Lochows Yellow, Hede, Gophers and Lochows × Victory, used in the previous experiments were sown on plots measuring 1 × 10 metres, at a drill spacing of 12 cm., for the purpose of more accurately measuring the yield after recovery from attack, to check any data obtained from the later sowings made in the previous experiment. Sowing was carried out with a Pragner drill, having four coulters, at a field rate of sowing (180–200 kg. per hectare). Five replications of each series were made, but Golden Rain and Gophers were omitted from the second series owing to lack of suitable ground. By June 22nd the first sown series was in the late three-leaf and early four-leaf stages and the plants were tillering, while the later sown series was showing through. By June 29th the first series was in the late four- and early five-leaf stages and the later sown series in the early two-leaf stage. The first series of plants therefore passed through its period of susceptibility when the flies were in their maximum prevalence period, but the second series, although equally susceptible, was exposed only to a decreasing population. Samples, comprising the plants in 1 metre length of drill, from one drill of each variety

Table X.

Large comparative trial. Percentage infestation with standard error.

Variety in order of sowing	Percentage infestation with standard error		Difference in percentage infestations compared with Victory	
	First sowing	Second sowing	First sowing	Second sowing
Victory	34·7 ±2·2	19·4 ±2·3	—	—
Golden Rain	34·6 ±2·3	Not sown	− 0·1 ±3·2	—
Black Bell II	22·0 ±2·1	7·7 ±1·5	−12·7 ±3·0	−11·7 ±4·3
Lochows	26·6 ±2·0	8·7 ±1·7	− 8·1 ±2·9	−10·7 ±3·7
Hede	11·9 ±1·3	11·5 ±1·8	−22·6 ±2·5	− 7·9 ±2·7
Gophers	30·8 ±2·2	Not sown	− 3·9 ±3·1	—
Victory × Lochows	22·7 ±2·0	9·2 ±1·6	−12·0 ±2·9	−10·2 ±3·7

throughout the series, were taken on July 11th and 12th and analysed, the results being recorded in Table X.

The heavier infestation of the first sowing is explained by the fact that only this series was exposed to any considerable number of flies. It is interesting to note that both Golden Rain and Gophers again were not any more resistant than Victory, while Bell II, Lochows, Hede and Lochows × Victory proved to be superior in each series. Of these four varieties, Hede was significantly more resistant than the other three in the first series but it was not differentiated in the second series.

It is somewhat doubtful whether the yield data from these experiments will have any value as the crops suffered very much from moisture during maturation.

2. *Data from observation plots.*

Large numbers of different varieties of oats are sown annually at Svalöv for observation purposes only, and examination of some of these varieties was rendered possible by the courtesy of Dr Åkerman. These varieties were sown on metre plots at the normal board spacing and they reached the four-leaf stage by June 16th, their susceptible stages therefore coinciding with the increasing prevalence of the fly. The plots were sampled on July 7th in two places, namely, at the extreme edges of the plots, the minimum, mean and maximum numbers of plants and shoots per sample being 15, 24, 30 and 47, 106, 167 respectively. The sampling was necessarily restricted by the fact that the material was required for other purposes and the results should only be considered in conjunction with those obtained from other sources. In estimating the infestation the samples have been taken together and the standard errors worked out on the basis of the number of shoots per sample. These data are shown in Table XI.

All these varieties except Nidar were actually less infested than Victory, but, as may be seen by reference to Table XI, the observed differences were not significant in the case of ten of them. Of the fifteen varieties, significantly less infested than Victory, twelve were less infested to the extent of 10 per cent. and above, but none of these differences, which range from − 12·0 to − 19·7 per cent., were significant, therefore these varieties, which include Lochows × Victory, Lochows, Lochows × Golden Rain and Roslags, cannot be differentiated amongst themselves on this evidence. The general trend of these data confirmed the results of the previous variety trial. Only five were infested to the extent of 5 per cent. above Lochows × Victory, the varieties from Norrbottens

Table XI.

Observation plots.

Swedish ref. no.	Variety	Percentage infestation with standard error	Difference in percentage infestation compared with Victory	Standard error of difference	Measure of significance
861	Victory	27·9 ±2·1	—	—	—
868	Abeds Nova × Victory	23·7 ±2·7	− 4·2	3·4	1·22
876	Lochows × Victory	9·2 ±1·8	− 18·7	2·8	6·70
877	Another line of above	10·4 ±2·1	− 17·5	3·0	5·84
878	Lochows × Golden Rain	8·2 ±1·8	− 19·7	2·8	7·12
879	Lochows Yellow	13·8 ±2·4	− 14·1	3·2	4·41
882.9	Yellow Flanders	14·9 ±2·7	− 13·0	3·4	3·70
882.10	French Yellow Giant	9·8 ±2·4	− 18·1	3·2	5·69
884.3	Walstedt (Bell II)	18·9 ±2·9	− 9·0	3·6	2·50
884.6	Another line of above	19·6 ±2·7	− 8·3	3·4	2·44
884.7	„ „ „	15·9 ±2·3	− 12·0	3·1	3·86
884.9	Engelbrekt	14·2 ±2·3	− 13·7	3·1	4·44
884.13	Roslag × Bell II	8·8 ±1·9	− 19·1	2·8	6·72
884.15	Argus	22·5 ±3·3	− 5·4	3·9	1·37
884.16	Bell III × Golden Rain	11·7 ±2·0	− 16·2	2·9	5·52
884.22	Roslags	12·0 ±2·0	− 15·9	2·9	5·47
884.25	Wisingsö	18·3 ±2·5	− 9·6	3·3	2·92
886.5	Ex Angermanlant	15·5 ±2·3	− 12·4	3·1	3·95
886.6	Thor	21·3 ±3·6	− 6·6	4·2	1·57
886.9	Nidar	28·8 ±3·8	+ 0·9	4·3	0·21
888.9	Mesdag	18·9 ±3·5	− 9·0	4·0	2·22
888.10	Norrbottens	25·3 ±3·2	− 2·6	3·8	0·68
888.11	Another line of same	18·3 ±2·8	− 9·6	3·5	2·74
892.1	Mulga (N.S.W.)	21·9 ±3·2	− 6·0	3·8	1·58
895.1	Joanette	24·7 ±2·4	− 3·2	3·2	1·00

and Wisingsö alone showing significantly less resistance than Lochows × Victory.

3. *A Lochows × Victory crossing and its results.*

The Director of one of the Svalöv sub-stations made the crossing between Lochows, the common German yellow oat and Victory, and found one or more of the lines therefrom to give promise of higher yielding capacity than Victory. Hence it was brought under observation at Svalöv, found to maintain its yielding capacity and is now being multiplied for commercial purposes, probably reaching the market in two years time. Dr Åkerman thought that, by visual observation, he could distinguish a difference in resistance in spring between Lochows and Victory, and it was a matter of considerable interest to determine whether the

cross Lochows × Victory would inherit the resistance of Lochows, if the latter were proved to be resistant also.

Five sets of data relating to the resistance of these three varieties have been examined and a weighted mean difference in percentage attack, as compared with Victory, obtained for both Lochows and the cross, Lochows × Victory, from four of the sets, in which the estimation of the percentage infestation has been based on the total numbers of shoots present on the plots. The collected data are shown in Table XII, except for the data provided by the primary shoots of the second sowing in the variety trial, which indicated a difference in percentage infestation, as compared with Victory, of the order of $-17{\cdot}5 \pm 3{\cdot}4$ for Lochows Yellow and $-19{\cdot}7 \pm 3{\cdot}3$ for the cross Lochows × Victory.

Table XII.

Difference between percentage infestations as compared with Victory.

	Variety trial			
Variety	First sowing total shoots	Second sowing total shoots	Comparative trial total shoots	Observation plots total shoots
Lochows Yellow	$-11{\cdot}9 \pm 1{\cdot}9$	$-9{\cdot}0 \pm 2{\cdot}2$	$-8{\cdot}1 \pm 2{\cdot}9$	$-14{\cdot}1 \pm 3{\cdot}2$
Lochows Yellow × Victory	$-12{\cdot}2 \pm 1{\cdot}8$	$-14{\cdot}4 \pm 2{\cdot}0$	$-12{\cdot}0 \pm 2{\cdot}9$	$-17{\cdot}5 \pm 3{\cdot}0$

The weighted mean difference in percentage infestation was $-10{\cdot}7 \pm 1{\cdot}2$ for Lochows Yellow and $-13{\cdot}6 \pm 1{\cdot}1$ for Lochows × Victory, the significance of the difference between these two being of the order of 2·25 ($D = 2{\cdot}9 \pm 1{\cdot}29$). The result is of interest because it shows that valuable economic results may be the outcome of such investigations.

VIII. UTILISATION OF OBSERVATIONS ON RESISTANCE.

The object of the previous experimentation was of course to discover the varieties most resistant, in both senses of the term, to the attack of the frit fly. Certain information having been acquired the next step was to utilise this information for building up a heavy yielding resistant variety, because the heavy yielding varieties themselves unfortunately occupy low positions in the scale of resistance. The work of crossing had to be carried out immediately after the analysis of the shoots, namely about July 20th onwards, and in consequence it was possible only to glean a very general idea, from the mass of recorded but unanalysed data, as to which were the most resistant varieties. As will be seen by reference to Table XIII ten of the varieties showing some evidence of superiority over Victory were crossed with Victory, Golden Rain II and Star.

Table XIII.

Varieties used for breeding, showing numbers of panicles, each of which carried 20–25 spikelets, utilised for crossings.

	Victory	Golden Rain II	Star	Dala	Ligowo
Lochows × Victory	5	5	.	.	.
Lochows	5	4	5	.	.
Lüneburger	5		.	.	.
Hede	12	15	5	3	.
Spet	13	10	5	.	4
Leutewitzer	4	.	4	.	5
Dala	5	.	5	.	.
Summer	4	.	2	.	.
Roslags	5	.	5	.	.
Kytö	.	5	5	.	.

This breeding work is at present purely empirical, no evidence whatever being available as to what characters may be associated with resistance. From previous work it seems likely that the fourth-leaf stage is the critical stage of the growth period of the plant, when the rapid elongation of the internode may bear the growing point out of reach of the minute larva or when the renewal of growth after tiller formation may induce unfavourable conditions for the larva, due to biochemical changes in the sap itself. Changes or differences in such characters or in physical characters of the tissues may account for the differences in resistance shown by the different varieties. In the absence of any such illuminating facts and without much precise information as to the reality of observed differences, it was only possible to make crosses somewhat at hazard and hope that some of the progeny would show both desirable characters, namely high resistance and high yielding capability. Hede and Spet were used for making numerous crossings with Victory and Golden Rain II because (1) of the notorious difficulty associated with seed-setting in oats after artificial pollination, 2 or 3 per cent. being the normal order of success in Sweden, and (2) of the desirability of attaining some success with these two varieties. Without the able and willing assistance of the technical staff of Dr Åkerman it would not have been possible to complete this work, because it involved the fertilisation of approximately 3500 flowers, carried on about 150 panicles. The progeny of these crosses were sown as early as possible in the spring of 1928, to obtain from them the maximum numbers of seed. The F_2 generations will then be sown late in the spring of 1929, and after natural selection by the fly has eliminated the

less resistant types, desirable types will be selected for multiplication for further examination by yield trials.

IX. APPENDIX. A SECOND ANALYTICAL METHOD APPLIED TO THE DATA.

As this analytical method may not be considered applicable by some, the data have also been analysed by the method which utilises the average value of the variance, obtained by considering all possible pairs of varieties (8). The standard error of the mean difference in percentage infestation between any two varieties, for each of the series except the primary shoots of the first sowing, is shown in the following table:

	Standard error of mean difference	Standard error × 2·57
First sowing, primary shoots	Insufficient data	—
First sowing, total shoots	4·08	10·5
Second sowing, primary shoots	6·38	16·4
Second sowing, total shoots	4·77	12·3

The standard error, multiplied by the figure 2·57, gives a value which may be used to differentiate between any two records in each series tabulated in Table III. Percentage infestations differing by more than these values, within the respective series, are significantly different. This method of presentation, therefore, makes it very simple for anyone to determine the comparative position of any variety. In general, of course, the two methods give equivalent results, but in this case the differentiation is not so fine.

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NOTE ON THE GROWTH OF YOUNG MICE SUCKLED BY RATS

By A. S. PARKES, D.Sc. (*Beit Memorial Research Fellow*).
(*From the Department of Physiology and Biochemistry, University College, London.*)

In a previous paper (Parkes(1)) it was shown that the growth of young mice during the suckling period was inversely proportional to the number suckling, and all the evidence suggested that this differential growth probably depended on the fact that each member of a large litter received less nutriment than one from a small litter. The growth curves of the individuals of the small litters (one and two) were extremely steep, and resulted in relatively enormous animals at weaning time.

In view of these results it was clear that it would be of interest to provide an unlimited milk supply for even a large litter and thus to ascertain:

(*a*) Whether the explanation put forward previously relating to differential nutrition could be substantiated by showing that the difference in the growth curves of large and small litters disappeared under conditions of unlimited nutrition.

(*b*) What ultimate degree of steepness in the growth curve could be attained by unlimited nutrition.

It was thought possible that such conditions of practically unlimited nutrition could be obtained by foster-mothering young mice on to lactating rats. The lactating rat produces enough milk to allow of the growth of five to seven young rats from 5·0 gm. at birth to 30 gm. at 3 weeks, *i.e.* to allow of the production of 100 to 150 gm. live weight. This amount would enable mice to reach adult weight by weaning time, and would represent, therefore, practically an unlimited nutrition for the smaller animal. This idea of rearing young mice on lactating rats was found in practice to be feasible, but the manœuvre required much care in selecting docile rats, etc., and even when the mice were not eaten, many difficulties arose, such as the weight of the rat when lying on the young mice and the large size of the nipples. In spite of all this, however, sufficient success was attained to make it possible to collect the following data.

Seven litters of mice in all were reared solely or partially on rats. The table shows the age at which the transfer to the rat was made, and the size of the litter, together with the average daily weight.

Growth of young mice suckled by rats.

No. of litter		GM 1	GM 2	GM 3	GM 4	GM 5	GM 6	GM 7	Normal for mice
Size of litter		5	2	3	4	5	7	6	—
Day put on to rat		10	At birth	10	7	3	9	2	—
Average weight in gms. per days old	0	—	2·1	—	—	1·9	1·6	1·8	1·41
	1	—	2·2	—	—	2·2	2·1	2·2	1·64
	2	—	2·5	—	—	2·4	2·3	2·5	1·90
	3	—	3·5	—	—	2·8	2·7	2·9	2·20
	4	—	4·0	—	—	3·0 (4)	3·0	3·5	2·53
	5	—	5·0	—	—	3·4	3·4	4·2	2·85
	6	—	5·7	—	—	3·9	3·7	4·8	3·19
	7	—	6·5	—	4·1	4·4	4·1	5·3	3·54
	8	—	7·2	—	4·8	5·0	4·4	5·8	3·86
	9	—	8·2	—	5·5	5·6	4·6	6·4	4·15
	10	4·6	9·0	6·3	6·2	6·4	5·2	7·2	4·43
	11	5·4	9·7	6·8	7·1	6·9	5·6	7·8	4·66
	12	6·4	10·5	7·4	8·0	7·4	6·2	8·4	4·85
	13	7·2	11·5	7·8	8·5	8·0	6·5	9·0	5·04
	14	7·7	12·2	8·3	9·0	8·5	6·9	*	5·20
	15	8·2	12·8	9·0	9·6	*	7·3	—	5·33
	16	9·0	13·5	9·4	10·1	—	7·5	—	5·48
	17	9·4	14·0	9·9	10·7	—	7·7	—	5·71
	18	9·9	14·7	10·3	11·3	—	8·0	—	6·00
	19	10·9	15·5	10·8	12·0	—	8·3	—	6·35
	20	11·3	16·1	11·5	12·7	—	8·5	—	6·72
	21	11·8	17·0	12·2	13·5	—	8·9	—	7·14

* Overlaid by rat.

From this table it will be seen that where only two young were put to the rat the phenomenal average weight of 17·0 gm. was attained by 21 days old. This increase in weight is nearly double that found in litters of two suckled on mice (Parkes(1)), and some idea of its magnitude can be obtained by considering that anything between 50 and 100 c.c. of milk each must have been consumed in the 3 weeks. This great increase in size, however, did not cause a corresponding increase in development (the eyes, for instance, opened at the normal time—13 days old), and the animals at 3 weeks old were practically immobile owing to the failure of the immature frame to cope with a weight almost equal to that of the adult animal.

Owing to their increased number and to being put to the rats later the other litters failed to show quite as surprising a growth as was found in GM 2, but in every instance the growth found was far in excess of that normal for the size of litter in question. The correlation between

number suckling and growth rate is not found in these mice suckled by rats.

From these results it is possible to conclude:

(*a*) That the variation in the growth of the various sizes of litter in the normal mouse is purely a question of differential nutrition.

(*b*) Under conditions of unlimited nutrition the growth of young mice may proceed to a degree which is both unusual and unhealthy.

REFERENCE.

(1) Parkes (1926). The growth of young mice according to size of litter. *Ann. App. Biol.* xiii.

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REVIEWS

Filterable Viruses. By T. M. Rivers. London: Baillière, Tindall and Cox, 1928. Pp. ix + 428. 15 Plates and 26 Figures. Royal 8vo. 34*s.* net.

Virus diseases are known to occur in man and other mammals, in birds, insects, plants, even, it is probable, in bacteria. It is no small labour merely to discover all the papers dealing with a subject whose matter covers so large a field and is so distributed, and the literature is now so vast that even the specialist can hardly keep in touch with all its ramifications. Yet a stray fact definitely established in any one field, however remote, may at any time throw a light upon obscurities in all the others. A book, therefore, which brings together in a general survey the information accumulated in regions so diverse is sure of a welcome. No general survey, of course, can be expected to give a complete analysis of all the work that has been done on virus diseases, and even in this volume of over 400 pages the treatment has been simplified by the selection of representative diseases within the various groups. Thus we have separate chapters on the virus diseases of man as exemplified by poliomyelitis, of mammals as exemplified by foot-and-mouth disease, of birds by fowl-pox, followed by chapters on the virus diseases of insects, of plants and of bacteria. With the possible exception of bacteriophagy, these diseases would be generally accepted as genuine virus diseases, but no satisfactory answer can be given to the question "what *is* a virus disease"? There are at present no definite criteria by which to decide whether any particular disease is or is not due to virus. Even the filterability of the causal agent—and the title of this book is *Filterable Viruses*—does not sharply differentiate the group, because on the one hand this character is shared by some bacteria, vibrios, spirochetes and protozoa, and on the other in some admittedly virus diseases, *e.g.* chicken-pox, no filtration experiments are recorded; and in others, *e.g.* vaccinia, the agent is either not filterable at all or filterable only with the greatest difficulty.

The volume begins with a chapter on "Some General Aspects of Filterable Viruses" by Dr Rivers. In this chapter (which has already appeared in the *Journal of Bacteriology* in substantially its present form) the author enumerates the outstanding problems which the study of virus diseases has raised, immunity, size and filterability of the agents, specificity, influence on cells and the like, summarises briefly (too briefly, we think) the information available under each head, suggests some lines for future work, and concludes with a summary of seven lines. In our opinion this chapter might well have been expanded considerably. Dr Rivers has restricted himself very largely to a statement of the various conflicting views and a conclusion that this or that question has not been satisfactorily settled. We should have welcomed a discussion on broader lines, especially since the summary here given is already in print and available for most workers. There is a number of aspects, barely or not at all touched upon, that might well have received consideration, *e.g.* the consequences that must follow from an intracellular habit of life as contrasted with the extracellular habit of most bacteria, the existence of toxins, the possibility of origin *de novo* (Rous's tumour).

This first general chapter is followed by one on filters and filtration by Prof. Stuart Mudd, a very useful chapter, and a salutary because an astounding quantity of nonsense has been deduced from observations on filtration. This is a good chapter. No mention is made of d'Herelle's method of sterilising membrane filters. A very interesting discussion by Carrel of tissue-culture in the study of viruses follows; and after this there is a cautious chapter by Dr Cowdry on intracellular pathology with some excellent illustrations of cell-inclusions, and a judicious discussion of the possibility of drawing conclusions from the available data. Then follow the chapters already

mentioned on typical virus diseases. Each of these is by a different writer, of authority on the subject with which he deals. No doubt the specialist on each will find points to disagree with and statements he is not ready to accept; but each is well done and gives an excellent presentment of its subject.

On the whole a very useful book: sometimes very good and occasionally stimulating.

J. HENDERSON SMITH.

(1) *Manual of Plant Diseases.* By F. D. HEALD. McGraw-Hill Publishing Co., Ltd., 1926. Pp. xiii + 891. 272 Text-figures. Price 35*s.* net.

(2) *Principles of Plant Pathology.* By C. E. OWENS. New York: John Wiley and Sons, 1928. Pp. xii + 627. 222 Text-figures. Price 23*s.* 6*d.*

(3) *Comparative Morphology of Fungi.* By E. A. GÄUMANN. Translated and revised by C. W. DODGE. McGraw-Hill Publishing Co., Ltd., 1928. Pp. xiv + 701. 406 Text-figures. Diagrams XLIII. Price 37*s.* 6*d.*

The past decade has been notable in plant pathology for the number of textbooks which have been published—in Germany a new edition of Sorauer and volumes by Höstermann and Noack, Riehm, Kirchner, Neger, Morstatt, Köck and Fulmek. Graebner, etc.; in France volumes by Marchal, Mangin, Nicolle and Magrou and new editions of Delacroix and Maublanc, Bourcart, etc.; in Sweden a new edition of Eriksson; in Spain a textbook by Gonzalez; in Czecho-Slovakia one by Smólak; in Russia volumes by Naoumoff and Bondarzew; in Japan treatises by Suematu and Ideta; in various parts of the British Empire books by van der Bijl, Cunningham, Butler, Nowell, Petch, Dade and Bunting, Bewley, Fryer, etc.; in the United States volumes by Stevens and Hall, Taubenhaus, Hesler and Whetzel, Chupp, Harshberger, Smith, Fawcett and Lee, Rankin, Mason, Anderson and Roth, etc., and the present authors Heald and Owens. Of the above some are general works, whilst others deal with one or other aspect of plant pathology but they are all alike in being of the nature of textbooks.

This fecundity is partly due to post-war reaction, but, even so, no science can reproduce so prolifically unless it has reached years of maturity. Plant pathology, which is so largely the child of academic botany, has as a matter of fact come of age. As a science it is showing increasing independence of its parent; founding its own professorial chairs, its own teaching schools, its own research institutes and its own committees of management. Although linked to botany by filial ties and common interests, yet it has its own life to live and its own contribution to make to knowledge and to human welfare. In America the separation has become almost complete; in England it is admitted almost less than in any other country and the parental authority of academic botany is still greatly in evidence. The present volumes may make these movements a little clearer to the older fashioned botanists and perhaps induce a more understanding and sympathetic attitude of mind to what is an inevitable course of development.

A second point of general interest arising out of these books and one that is ripe for discussion, especially in England, relates to the scope of plant pathology. Prof. Owens appends to his chapters lists of "Review Questions," and one of these asks "What is the difference between 'plant pathology' and 'mycology'?" Many students in this country do not recognise any difference and point to the fact that in England "plant pathologists" are in almost all cases officially termed "mycologists." On the other hand, a few years ago a distinguished American student wrote "Until very recent years plant pathology has been considered as simply a phase of botany or as applied mycology. A brief course in mycology masquerading under the name of plant pathology has in most cases sufficed to dispose of the subject. Even the so-called

plant pathologists of the present day are in large part only mycologists with little of the true phytopathologic point of view."

Heald answers the question by describing his book as "An attempt...to present a view of the whole field of plant pathology including environmental and virus diseases as well as those of bacterial and fungous origin, as it is felt that a book of restricted scope would perpetuate an erroneous notion which has been prevalent in recent years as to the real province of plant pathology." The latter he defines as "The consideration of all non-parasitic diseases, the virus diseases, all troubles due to the five groups of plant parasites, and in addition...those due to nematodes and also those of protozoan origin." The above sentences might equally well have been quoted from Owens' treatise.

Until plant pathology secures autonomy on this wide basis and takes rank with such independent disciplines as human and veterinary medicine, the science as a science will make little progress. The English titles of "Mycologist" and "Mycology" give a false idea of subordinate value and dependence, once perhaps true, but now erroneous and stultifying and subversive of the best interests of plant pathology. The equation of mycology with plant pathology has in addition a serious adverse influence on the study of mycology itself, for attention is deflected from pure research on the fungi to more economic ends. Mycology is the study of fungi *qua* fungi and plant pathology is the study of plant disease *qua* plant disease whatever its etiology, state or relations. Their viewpoints are fundamentally different and their only contact is that a certain considerable area of their provinces is held in common just as happens with veterinary medicine and physiology or human medicine and bacteriology. The difference between the sciences is well shown by a comparison of the volume by Gäumann and Dodge with those by Owens and Heald.

Gäumann and Dodge's book is an account of the structure and development of the fungi written to give understanding of these organisms as living things with intrinsic value and interest of their own, irrespective of any economic relationships they may show with other organisms, their inanimate environment or matters of human welfare. The fungi are studied for their own sake as one studies the algae or the pteridophytes.

The volumes by Heald and Owens are concerned with disease in plants; the nature of disease, the agencies which bring about disease, the losses to man caused by disease and the prevention and cure of disease. In Heald's book 58 pages are given to history and symptomatology, 175 pages to non-parasitic diseases, 55 pages to virus diseases, 63 pages to bacterial diseases, 19 pages to myxomycetous diseases, 440 pages to fungous diseases, 21 pages to phanerogamic diseases and 22 pages to nematode diseases. In brief about one-half the volume deals with diseases caused by fungi, but even here the diseased host is the centre of attention and not the fungus, the latter only being of interest in so far as its discussion contributes towards an understanding of the diseased state. In fact most of the pages dealing with fungous diseases are filled with descriptions of the history and distribution of the specific diseases, their symptoms, their effects and their economic importance; with discussions of predisposing factors, host relations, control measures, etc., the fungi themselves being little more than mentioned or briefly described.

The same construction appears in Owens' book. The first 157 pages are devoted to generalities concerning disease and its relation to environmental factors, 13 pages to myxomycetous diseases, 54 pages to bacterial diseases, 276 pages to fungous diseases, 16 pages to phanerogamic diseases, 20 pages to nematode diseases, 50 pages to virus diseases and 24 pages to various non-parasitic troubles. Here again the two-fifths of the book dealing with fungous diseases only treats of the fungi in so far as they are necessary to understand the diseased crop plant and most of the text deals with host symptoms, economic aspects and control measures.

If these books do anything to undermine the domination of plant pathology by academic mycology and so help to destroy what is clearly an untenable and obsolete position they will have done yeoman service.

A few words may be said in more direct appreciation and criticism of the volumes. For many years there has been great need of a major general textbook in English

on plant disease. Owens' volume was, apparently, first issued in mimeograph form in 1924 and has just been published in its present state, whilst Heald's book was issued in the winter of 1926–7.

Principles of Plant Pathology by Prof. Owens is essentially a textbook for the American undergraduate student and to each chapter are appended directions for laboratory study, "review questions" and extensive bibliographies in which the references are almost exclusively American. In fact, the whole volume is lacking in appreciation or even mention of non-American work. This is illustrated for example by the lists of textbooks and scientific journals given on p. 155. Only eleven journals are mentioned, of which eight are American, two are English—*Annals of Botany* and *Annals of Applied Biology*—and one is German—*Zeitschrift für Pflanzenkrankheiten*. Adequate comment is not easy. The actual diseases are on the whole well described and illustrated, and a student working conscientiously through the volume would emerge with a fairly sound knowledge of American plant diseases. If he then proceeded to a European laboratory to widen his experience and obtain a truer perspective he might become a sound plant pathologist.

The *Manual of Plant Diseases* by Prof. Heald is a textbook for students of better quality. The difference between the volumes by Owens and Heald is exemplified in the bibliographies appended to the chapters. Taking one at random, that on Downy Mildew of Grapes for example, Owens gives five name references—all American—whilst Heald gives fifteen, of which only two are American. The textbook by Owens is parochial in outlook, that by Heald much more cosmopolitan. In spite of minor detractions in Heald's book such as obvious misprints and somewhat trival headings —"pondscum parasites," etc., which look rather silly in a treatise of this calibre— the volume is a serious work of considerable value.

When Prof. Gäumann's *Vergleichende Morphologie der Pilze* was published in 1926 it was at once recognised as a work of outstanding importance. Since De Bary's volume 40 years previously no comprehensive treatise on this subject had appeared and the need was urgent, for in the interval the study of fungus morphology had made immense strides. The advance was almost entirely a filling in along lines largely foreshadowed by De Bary, and it has been concerned chiefly with the cytology of fungus reproduction, mycologists out-Freuding Freud in their obsession with sex. Gäumann brought together in a masterly way an amazing amount of data, his volume containing fine illustrations of a large number of fungi, and referring explicitly to species whose mere names comprise nearly all of 30 double-columned pages of Index.

The American edition is not a simple translation, for Prof. Dodge has incorporated the new literature appearing between 1925–7 and rewritten certain sections. The Ascomycetes and Basidiomycetes particularly have been reconsidered and one might perhaps instance the treatment of the Gasteromycetes or of the Laboulbeniales as striking improvements. But, *O tempora! O mores!* For 30 years students have observed almost with reverence the spermatia caught *flagrante delicto* on the trichogyne in Thaxter's figures of *Stigmatomyces Baeri* and now in a mere footnote we are told that these are not spermatia at all but only receptive prominences of the trichogyne itself!

In the American edition there are many minor alterations; the omission of the list of general books, correction of errors in synonymy, relegation of authorities to the Index and the assemblage of the several bibliographies in a single chapter of 40 pages at the end of the book. An unfortunate alteration is the introduction of numerous misprints. When one reads for example that the Ancylistaceae and the Pythieae are both "partially endosparaltic" it takes one a moment or two to decide that this is merely a misprint of "endoparasitic" and not still another technical term; and this kind of thing is too common in the volume.

Prof. Gäumann's original work is a little heavy going due to his strict adherence to technical terms, and the new edition is certainly no improvement—"The chiastobasidial Autobasidiomycetes have attained the same degree of development in this family as the stichobasidial Phragmobasidiomycetes in the Septobasidiaceae and the lepto-forms of the Uredinales"—and so on for 700 pages. Throughout, there is hardly any lightness of touch, and when, on page 288, the authors, speaking of the transitional

forms in the Sphaeriales remark, parenthetically, that these are "inconvenient for systematists" or on p. 455 describe the Agaricales as "of fatiguing regularity" one is inclined to smile as at a huge joke.

The aim of the original work is well stated on p. 427 as "not to make easy the recognition and identification of an unknown fungus but to discuss fundamental questions of relationships and to point out gaps in our knowledge" and this purpose it fulfils admirably.

In the American edition Prof. Dodge has preserved many of the theoretical discussions of phylogeny, although he states in his preface that "it is impossible for me to agree with some of the conclusions." The whole structure of the work is based on certain phylogenetic concepts which are reviewed in the final chapter. The detailed applications of these are worked out for each section in turn and one must admire the way in which the original author kept in his mind the logical and processional sequence of values necessary for his constructions. The several moieties are crystallised out in numerous phylogenetic diagrams, and although one cannot here discuss these in detail one may perhaps express a personal opinion that the treatment is too didactic and exhibits a confidence in phylogenetic speculation which at times is a little astonishing. To describe the origin of any one genus of fungus from any other as "very evident" (p. 282) certainly gives one to pause. On a more general point one may wonder whether a two-dimensional diagram can in any way represent evolutionary sequence of a three-dimensional order.

Still, no book of this size and quality could please everyone and much of the fun of scientific research would disappear if we all lay down as lambs together. One can only be grateful to Prof. Dodge for his courage and skill in carrying out so onerous a task and assure him that botanical students throughout the English-speaking world will rise up and call him blessed.

WILLIAM B. BRIERLEY.

Leaf-Mining Insects. By James G. Needham, Stuart W. Frost and Beatrice H. Tothill. London: Baillière, Tindall and Cox, 1928. Pp. viii + 351 and 91 Figures. Price 27*s.* net.

In their prefatory remarks the authors mention that the object of this book is threefold and aims at providing (1) a non-technical introduction to the general subject of leaf-mining insects; (2) an account of their biology sufficiently detailed to be of value to the ecologist; and (3) lists of miners and their host plants and of the principal papers on these insects.

Sufficient is known of leaf-miners to-day to show that their study affords many problems of interest to the general biologist and the specialist alike. With the present volume, along with Dr Martin Hering's excellent *Ökologie der blattminierende Insektenlarven*, the subject is brought well up to date with copious bibliographies.

Four orders of insects, viz. Lepidoptera, Coleoptera, Diptera and Hymenoptera, include leaf-mining species. In all cases it is the larva which betrays this habit which is undeveloped in the adults. In the first two chapters of the book before us there is an elementary general discussion of the types of leaf-mining larvae, the moulding effects of similarity of environment on larval forms of diverse groups, the characters of the mines themselves, the origin of the leaf-mining habit and the subject of host preference. A classification of mines is given and a general table for separating larvae of the four orders already mentioned. There are also brief remarks on collecting and rearing, and a short discussion of species of economic importance. Chapter III is concerned with Lepidopterous leaf-miners in general and the eight chapters which follow each deal with a separate sub-division of the order. The great development of the mining habit in the order is reflected in the fact that one-half of the book is devoted to these insects. The various grades in specialisation of the leaf-mining habit and the various modifications in the structure of the head-capsule and mouth-parts are of interest from the evolutionary standpoint, which the work of Trägardh in Sweden has so excellently demonstrated.

Chapter XII deals with Coleoptera, an order wherein the leaf-mining habit is little developed. Chapter XIII is concerned with Hymenoptera where the habit is confined to the family Tenthrendinidae and Chapter XIV deals with Diptera.

On the whole the book serves as a valuable introduction to the subject. It is especially useful to the field worker whether he be an ecologist or an economic entomologist, and will prove of very material assistance in the identification of the species met with. The lists of leaf-mining species (Chap. XV) and the hosts (Chap. XVI) are a special feature of the book, and the bibliography will serve as a useful guide for all who desire more detailed information.

Information on leaf-miners is now so extensive that it becomes impossible to deal, in a book of this compass, with every species concerning which some facts relating to their habits are available. The European forms are well treated by Hering, and the present volume is concerned more especially with the American species. We presume it is the authors' intention to thus limit their subject although it is not expressly mentioned in the Preface. If this conjecture be correct, it explains why some notable European species are neither mentioned nor listed. Its treatment is frankly elementary throughout and, having mastered this volume, the reader should be well prepared to study the work of Hering already alluded to. The book is well arranged, the printing good and the illustrations are, for the most part, clear and adequate. It is not free from typographical errors, but these are insufficient to detract materially from its value and it will find a useful place in North American entomological literature.

A. D. IMMS.

Modern Biology. By J. T. CUNNINGHAM. Pp. xii + 244. London: Kegan Paul, 1928. 10*s.* 6*d.* net.

A botanist cannot but feel a little aggrieved when a book dealing only with certain aspects of zoology and omitting almost all mention of plant life, is entitled *Modern Biology*. Even the sub-title which states that it is "A review of the principal phenomena of animal life in relation to modern concepts and theories" suggests a far more inclusive scope than the book possesses. The contents of the volume may be indicated briefly. Following an explanatory introduction, Chapter I is devoted to a consideration of mechanistic biology and neo-vitalism, and is largely an adverse criticism of Needham's essay in *Science, Religion and Reality*. The second chapter deals with metabolism, adversely criticising Johnstone's views on the nature of life in relation to entrophy expounded in his volume *The Mechanism of Life*. The next four chapters are concerned with evolution and heredity and the part played by mutation, hormones, functional activity and external conditions. The view-point is that of an avowed Lamarckian, the author of a book on *Hormones and Heridity*. These chapters contain accounts of Heslop Harrison's work on induced melanism in Lepidoptera, Guyer's experiments on induced eye defects in rabbits, the work of Tornier and Berndt on abnormalities in goldfish, Kammerer's experiments on Alytes, Salamandra and Proteus and Weldon's twenty-five year old researches on Carcinus. In the final chapter mind and consciousness are discussed.

The book is written in a stimulating but somewhat controversial and forthright manner. It is a little parochial in outlook and the general perspective of values indicates perhaps a slightly myopic vision as is illustrated by the too frequent mention of a particular famous surname when others associated with equally brilliant and more massive work remain unmentioned and the researches unnoticed. It is also written perhaps somewhat carelessly, as is evidenced by the confusion in Chapter I and Index of a clever son with a wise father. Still, Mr Cunningham says many things that have needed saying for some time—that biochemistry and statistics are not biology, and that the purely biological approach to the study of life, of form and structure, of growth, development, heredity and evolution cannot, so far as we can see at present, be replaced by the chemical or statistical approach. Concerning the latter the author writes: "There is a tendency in modern biology to suppose that when structures and functions have been measured and the results expressed in curves,

graphs, or mathematical formulae, the causes of them have been discovered." This might have been expressed more strongly, for the fashionable idolatry of statistics by many biologists may, unless controlled, become a dangerous tendency, doing more to confuse and distort issues than to clarify them.

In his last chapter, on mind and consciousness, Mr Cunningham is writing on a subject which is not entirely his own. Much of the chapter concerns McDougall's "Lamarckian" experiments on rats which are quoted with approval, whereas Hazlitt's destructive criticism of them published in the same journal a few months later is not referred to. In concluding, the author discourses at large of social tendencies and to many of us his views, although interesting, might be regarded as lacking in insight.

As a whole the volume is essentially a statement of Mr Cunningham's well-known position, that evolution and its cognate issues cannot be explained in terms of Mendelism, mutation and natural selection, but that the influence of the environment, of functional activity and of the internal secretions must be taken into consideration as primary factors.

The volume is interesting and very readable, but it is possible to regard it as special pleading in that it is written from a particular point of view. The latter, however, needs expression and demands more respect than it commonly receives. On the whole the book is perhaps just a little disappointing, for it is so good that one feels it might easily have been just a little bit better.

WILLIAM B. BRIERLEY.

PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

ORDINARY MEETING held at 2.30 p.m. on Friday, October 26th, 1928, in the Botanical Lecture Theatre of the Imperial College of Science and Technology, London. The Chair was taken by Dr J. WATERSTON, Vice-President.

The following papers were read:

I. "On the Biology of *Sirex cyaneus* Fabr. (Hymenoptera-Siricidae) and its parasites in Britain" by Mr R. N. CHRYSTAL, M.A., B.Sc. (Imperial Forestry Institute) and Mr J. G. MYERS, Sc.D., F.E.S. (Imperial Bureau of Entomology).

II. "Notes on a Fungus associated with *Sirex cyaneus*" by Mr K. St G. CARTWRIGHT, B.A. (Imperial College of Science and Technology).

Mr CHRYSTAL gave an account of some biological studies which have been in progress during the past two years on the Sirex woodborer *S. cyaneus* Fabr., and its two parasites, *Rhyssa persuasoria* L. (Hymenoptera-Ichneumonidae) and *Ibalia leucospoides* Hochenw. (Hymenoptera-Cynipidae).

The biology of the woodwasp has been chiefly studied in relation to its attacks on larch, which is probably its principal host tree. The main details of this are now almost completely known, and one of the chief points which the research has brought out is that this insect is a secondary pest from the standpoint of the living tree, and therefore rather an indicator of pathological conditions than the direct cause of them. It has been shown, for example, that trees may be fungus-attacked (*Armillaria*, *Fomes*, etc.) or suffer from the effects of bad soil conditions and yet remain unattacked by the woodwasp for some time. The biology of the parasites, especially the Ichneumonid *R. persuasoria* L., was studied with the primary object of ascertaining whether or not one or both species could be exported to New Zealand as an enemy of a closely allied species of woodwasp (*S. juvencus* L.) which has become established there.

In the work on *Rhyssa* a considerable amount of new data has been accumulated, especially concerning the egg-laying habits, a full account of which was presented for the first time.

The habits of the Cynipid parasite, *Ibalia leucospoides* Hochenw. were, previous to this work, almost completely unknown. A fairly complete record of its biology has been obtained during the past 2 years, the main features of which are as follows.

The parasite has been shown to have an extremely specialised egg-laying habit. The larval stages are now known to be markedly hypermetamorphic and a study of the morphology of these will prove of extreme value in deciding the systematic affinities of the sub-family *Ibaliinae*. Lastly, it has been shown that this parasite, hitherto considered a very rare species in Britain, is to be found prevalent in nearly all localities where *S. cyaneus* is of common occurrence.

The paper was illustrated by lantern slides.

Mr Cartwright said: Work on the association of insects and fungi has been confined, up to the present almost entirely, to the entomological side. Many of the details in regard to wood-inhabiting insects have been excellently summarised by Paul Buchner (1) in a recent publication entitled *Holznahrung und Symbiose*, which contains drawings showing the presence of symbiotic organisms. A paper of a more exhaustive character by Breitsprecher (2) has just come out. This is mainly a description of sections cut through the abdomens of species of *Anobiidae*, showing the presence of symbionts.

The classical example of an association between insects and fungi is that of the *Ambrosia* beetles. In this case the fungus is confined to a narrow zone round the galleries of the insect and is definitely transmitted by the ovipositing female; the fungus spores being excreted on to the egg coat. A simpler association was described by Möller in his studies of leaf-cutting ants which cultivate fungi for their food supply, here the fungus, *Roziites gongylophora*, is definitely carried by the queen in a special cavity, to the new colony. Other related ants cultivate a fungus on chewed-up wood.

Much indirect evidence about the subject has been obtained from the practical foresters, architects and others who have so frequently noted that insect and fungus attack appear to be connected, as to suggest that some direct association existed between them. In these notes attention will be confined to the association between wood-feeding insects and fungi. This may be considered under two headings: I. Association of an insect with an internal symbiont, *i.e.* the regular presence in the body of the insect of a foreign organism which is often confined to special glands. II. The regular occurrence of a fungus in the wood or tissues of the host in which the insect is living.

I. It is not intended to discuss at any length the question of internal symbionts, as most of the information so far gained has been on the entomological side. Suffice it to say that in most timber beetles and bark beetles which have been examined, bacteria, yeasts or fungi proper have been found to occur; as an example of these, *Endomyces* may be mentioned.

It has been suggested that symbionts will be found to be present in insects which feed on materials such as wood, which are not readily digestible, whilst these will not occur when the insect lives on substances that are easily absorbed. The conclusion drawn is that these symbionts enable the insects to break up the wood, etc., and make available for their assimilation these foodstuffs. It has been assumed that the insect alone is unable to do this. Chemists who have analysed beetle frass have stated that it appears to be unaltered wood. These results, however, need confirmation as it is difficult to suppose that neither the fungus nor the beetle have in any way altered the wood during its passage through the body of the latter. Such would mean that their relationship was in the nature of a mutual parasitism without any outside source of energy. It would seem probable that such alterations as occur are not detectable by the present methods of wood analysis.

The organisms usually occur either in some part of the gut, into the epithelial layer of which they may work their way, or they may be contained in special glands opening into the vagina, being transmitted to the egg coating either before or during oviposition. The larva in hatching from the egg eats part at least of the egg coating and thus becomes infected.

Unfortunately up to the present no proof, as far as I am aware, has been brought forward that the internal symbionts do really help in the breaking up of the wood and all attempts to obtain these insects free from the organisms have so far failed. Until the technique for achieving this has been worked out, it is by no means certain that the insect really is dependent on its internal symbiont. Should it be found that the insect can live independently of any symbiont it does not rule out the possibility that such insects may not require wood already altered by fungus action or that in some instances the insect larva may be entirely mycophagous.

Although the sterilisation of the insect appears to be necessary to furnish conclusive evidence one way or the other, another line of attack which would afford a certain amount of circumstantial evidence is that of isolating these symbionts from the insects and growing them on artificial media. The actual technique of isolation may prove difficult, but should not be insurmountable. If this could be done, it could at any rate be ascertained whether they were capable of breaking up any of the wood constituents. Up to the present material has not been available on which to attempt this work, but it is hoped to undertake it in the near future.

II. Association of insects with fungi living externally in the surrounding material.

From the examination, by means of sectioning, of much wood attacked by beetle, it has been ascertained that wherever larval tunnels are present, there invariably can fungus mycelium be found. Up to the present no case has come under my observation in which this did not prove to be true. In the case of bark beetles for example, the tunnels are often seen lined with fungus which in certain instances is a species of *Endomyces*. Schneider-Orellis found thick-walled fungus spores in the gut of the overwintering female; these germinated readily in the excrement of the insects. During the examination of some ash wood attacked by *Daldinia concentrica*, tunnels of *Hylesinus fraxini* were observed; these were seen to follow closely the black zone lines caused by the fungus. In old beams and furniture there would appear also to exist some connection between beetle attack and the presence of fungi. Furniture which had been in store for about 8 years was found to be attacked by beetle and an examination showed that fungus mycelium was present.

The moisture content in old furniture, beams, etc., may be as low as 7 per cent. and is rarely above 15 per cent. Such low moisture contents would appear to exclude the possibility of finding mycelium in a state of active growth.

The fungi obtained by cultural methods from such old wood, which is often very much broken up by larval galleries, cannot be assumed to be those primarily concerned. It is highly probable that in many cases the fungi perform their work prior to the insect attack and that the beetle larvae feed either on the altered wood or on the dead fungus mycelium or on both.

Many different fungi commonly present in wood, such as *Trichoderma lignorum*, *Torula* sp., *Penicillium* spp., etc., have appeared in such cultures taken from near beetle tunnels. So far my work on the association of timber-destroying beetles and fungi has been confined to the examination of beetle-attacked wood for the presence of fungus and to keeping in culture those fungi which are isolated frequently from cultures taken near the tunnels or from frass.

Only in two instances has a Basidiomycete been obtained. *Salix* wood, containing tunnels of *Xestobium*, was decayed throughout by *Fomes applanatus*, but the beetle attack had obviously come in later and there was no question of the fungus having been introduced by the insect. A *Xestobium* larva has since been placed on a pure culture of this fungus growing on malt agar and it has been alive for about 1 month in the culture, on which it feeds to a certain extent. The second case was also on *Salix*, from which, amongst other fungi, a member of the Cyphellaceae has been obtained. In several beetle-attacked oak samples sent for examination, a fungus with conidia of the *Septocylindrium* type has been found and a species of *Torula* was also present.

These fungi have often been observed in old oak which shows "golden" coloration; from this *Eidamia catenulata* has also been isolated on several occasions. It would be interesting to ascertain whether these richly coloured and much sought after specimens of old oak are more susceptible to insect attack than is the normal, as Prof. Groom (3) and Mrs Williamson (4) have shown that such coloration can be caused by fungus action.

Finally it may be mentioned that a fungus having the β type spore of a *Phomopsis* has been isolated by Mr Day and Mr Nutman from *Lyctus*-attacked wood. This has not yet been identified with certainty and may possibly prove to be a species of *Cytospora*.

To summarise, two explanations of the regular presence of fungus in and around beetle tunnels may be put forward: (1) the fungus already present grows more actively in the region of the beetle tunnels because the aeration is better there and because it derives nourishment from the waste products of the insect which may tend to raise the moisture content of the wood around the tunnel; or (2) the insects make their galleries in that part of the wood where there is most fungus mycelium and also they spread the fungus by infection carried on their bodies.

Probably both explanations hold good to some degree and the fungus once introduced finds the conditions surrounding the tunnel the most favourable for its growth.

We may conclude, tentatively, that beetles always prefer wood which contains fungus, because (1) they are better able to digest wood which has been attacked by the enzymes of a fungus, and (2) they derive part or all of their food supply from the actual mycelium of the fungus, dead or alive. It is not to be expected that any wood-destroying fungi will be found living and growing actively in wood with a moisture content as low as that usually found in old oak beams, etc., as they require in most cases a moisture content about that of fibre saturation point (28 to 30 per cent.). It would be interesting to ascertain the lowest moisture content at which the various fungi isolated from beetle-infested wood could grow, and also whether the moisture content of the wood in the neighbourhood of the galleries was increased by the metabolism of the insect.

Sirex cyaneus *and fungus association.*

The study, of which this is an account, was undertaken on the suggestion of Mr Chrystal after a meeting of this Society on March 23rd of this year, and the work is as yet quite incomplete; thus only tentative conclusions can be drawn as to the results.

Attention was drawn to the paper of Buchner in which it is stated that oidia of a fungus had been found in a gland or squirt at the base of the ovipositor in *Sirex*

gigas. These glands Buchner figures. They are paired and open out into the vagina, the oidia being extruded on to the eggs after they have left the ovaries. Furthermore, he had observed clamp connections, proving that the fungus belonged to the Basidiomycetes, though in his figures these are by no means convincing, as they are in no case complete, the septa being absent. Certainly one would suspect them of belonging to a Basidiomycete.

Slides prepared at Oxford were sent me for examination in April. These showed oviposition and young larval tunnels, and in every case a mass of mycelium was present in the wood around the vessels. The outer sculptured wall of an ovum was left *in situ* in one of the tunnels and showed hyphae around it. At the same time slips of larch wood with oviposition tunnels and young larval galleries were sent, which, on sectioning, showed mycelium of a similar kind with clamp connections conspicuously present. From an examination of further specimens, both of wood attacked by *S. cyaneus* and of that attacked by *S. gigas*, it would appear that a fungus mycelium belonging to a Basidiomycete is always present in the wood in which these larvae are living. Furthermore, this mycelium in every case seemed to have its focus around the galleries and was definitely attacking the wood, which showed numerous bore-holes. The moisture contents of one sample were determined and found to range between 35 per cent. and 48 per cent., a condition of the wood which would be favourable for the growth of these wood fungi. From the original samples, culture plugs were taken and a fungus which grew readily on a 2 per cent. malt or prune agar, was isolated. This mycelium soon produced numerous clamp connections and was moderately rapid in growth. The culture remained white for some months, but now colour is developing in some of them. They are of a soft downy texture. The same fungus was isolated from several different samples. So far the cultures have shown no sign of fruiting though undifferentiated segments of hyphae separate off and may act as oidia.

The small specimens from which the cultures were taken already showed fungus mycelium throughout, though it was more plentiful in the neighbourhood of the tunnels. One could not feel certain, therefore, that this wood had not contained fungus before the *Sirex* attack. However, in August, material was obtained which contained oviposition tunnels and eggs of about 2 weeks old. These were sectioned and in every case the same type of mycelium was seen having the tunnel as its focus of development, the mycelium rapidly diminishing in amount away from the tunnel. Sections from areas closely adjacent to these tunnels showed no trace of fungus; demonstrating clearly that this really had been introduced by *Sirex* during oviposition.

The extent of development of the fungus after 2 weeks was sufficient to suggest that the 5 weeks' interval between oviposition and the hatching would be enough to allow the fungus to make sufficient growth to assist in the nutrition of the larva.

A few dead female adults and a number of half-grown larvae were obtained in August, the majority of these were fixed and have been embedded for the cutting of serial sections. One of the females was dissected and the eggs removed from the uterus and examined under the microscope. A fine mantle of mycelium was seen in between, and surrounding the eggs closely. No clamp connections were seen in this mycelium.

A few of the eggs were placed in slants of 2 per cent. malt agar: these cultures became contaminated but sufficient could be seen in the mixed culture to show the

presence in one case of a Basidiomycete. Eggs were also extracted from oviposition tunnels made about 2 weeks previously; of these, ten were placed on 2 per cent. prune agar slants and six on malt agar one egg being placed in each tube. Out of the prune tubes three gave cultures of mycelium showing the same characteristics as that of the fungus originally isolated from the wood, and bore clamp connections; four remained sterile, and two gave contaminants. Out of the malt, two gave mycelium with clamps and three remained sterile. From the tenth prune tube and the sixth malt tube the eggs were removed and found still to show the mantle of mycelium as seen round the eggs dissected from a female *Sirex*[1]. No fungus has been found around the eggs in the ovaries; further dissections will have to be made to settle this point definitely. A cursory examination of a few larvae showed mycelium to be present in a disorganised condition as if partly digested, but mycelium which appeared undisorganised was also seen. It is thought probable, therefore, that this may remain in a resting condition through the pupal stage and be found in the adult as described by Buchner.

A newly hatched larva, removed from its tunnel, was placed on a culture of the fungus where it lived for 3 weeks apparently feeding on the fungus. One of the half-grown larvae lived for 3 months on cultures of this fungus. It was transferred from time to time to fresh cultures which it could be definitely seen to eat.

Identification of the fungus.

The culture of the fungus from *Sirex cyaneus* agrees with none of the fairly numerous type cultures which I keep for comparative purposes. No clue has so far been vouchsafed either in the finding of sporophores on the trees or in the type of rot produced. It is probable that identification will be secured when more comparative cultures are obtained.

In regard to *Sirex gigas*, the species with which Buchner chiefly deals, I have only had a few specimens from some larch props sent for another purpose.

From an examination of these samples which contained pupae and from which adults hatched out in the laboratory, it would appear that a similar association exists here also.

Conclusions.

To sum up, it appears that sufficient evidence of a circumstantial nature has been brought forward to show that:

1. A Basidiomycete fungus is always present in wood-containing *Sirex cyaneus*.
2. This fungus has proved so far to be identical in every case examined.
3. That the fungus is introduced with the egg during oviposition. In the cases examined this has been in the form of a fine mycelium which, being in the primary condition at the time of oviposition, has no clamp connections. It develops these, however, both in the wood and in artificial media.
4. The fungus causes a rot of the wood in which bore-holes are made.
5. It can advance sufficiently in the time elapsing between oviposition and the hatching out of the young larva either to have formed food for the larva itself or to have acted upon the wood to a sufficient extent to make this available as food.

[1] Some of the cultures which had remained sterile are now showing fungus development.

6. The larva can live and definitely grow on a pure culture of the fungus for a period of at least 3 months, showing that it can derive some nourishment at any rate from eating fungus alone. Further information is needed about the methods whereby the fungus is transmitted from the larval through the pupal stage to the adult, and at what stage in the life-history of the insect the special glands containing the fungus are formed.

Probably some stimulus due either to fertilisation or gland secretion, when the resting oidia are extruded from the gland into the vagina, starts growth.

In the case of *Sirex gigas* the fungus may also be introduced into the wood as oidia: details of this have still to be worked out. It is hoped next season to obtain cultures from eggs directly after oviposition and possibly even to induce egg-laying directly into a medium. Material was not available for this during last summer.

Finally, I wish to acknowledge the debt I owe to the previous speaker who started me on this work and who, with the skillful help of his assistant, has provided me with such excellent material.

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(3) Groom, Percy (1915). "Brown Oak" and its Origin. *Ann. Bot.* XXIX, No. 115, July.

(4) Williamson, Helen S. (1923). The Origin of "Golden Oak." *Ann. Bot.* XXXVII, No. 147, July.

The following letter has been received by the Editors of the *Annals of Applied Biology*:

Abol Research Laboratories,
Paddock Wood,
Kent.
October 1928.

Dear Sirs,

With reference to the article on "The use of Tetrachlorethane for Commercial Glasshouse Fumigation," which was published under my name in the May issue of your *Journal*, I regret that I failed to acknowledge that I was indebted for a considerable part of my information to Messrs Murphy and Son, Ltd., as a result of investigations which I made for them while engaged by them. I omitted to mention in the article that the introduction of tetrachlorethane into Commercial Horticultural practice by the writer in 1920 was in fact effected through Messrs Murphy and Son, Ltd., and was as a result of the investigations referred to above.

Yours faithfully,
(*Signed*) Theodore Parker.

The Editors,
The Annals of Applied Biology,
Cambridge University Press,
Fetter Lane, E.C. 4.

Ordinary Meeting of the Association held at 2.30 p.m. on November 23rd, in the Imperial College of Science and Technology, South Kensington. The President, Dr E. J. Butler, C.I.E., F.R.S., in the Chair.

I. "The Relation of Environmental Conditions to Angular Leaf Spot Disease of Cotton (*Bacterium malvacearum*)" by Mr R. H. Stoughton, B.Sc. (Department of Mycology, Rothamsted Experimental Station, Harpenden, Herts.).

II. "The Effect of Environmental Conditions on Plant Diseases under Glass" by Dr W. F. Bewley (Director, Experimental and Research Station, Cheshunt, Herts.).

III. "Temperature and Humidity in Relation to Tomato Mildew (*Cladosporium fulvum*)" by Mr T. Small, B.Sc. (Experimental and Research Station, Cheshunt, Herts.).

I. THE RELATION OF ENVIRONMENTAL CONDITIONS TO ANGULAR LEAF SPOT DISEASE OF COTTON (*BACTERIUM MALVACEARUM* E.F.S.).

By R. H. Stoughton, B.Sc., A.R.C.Sc.

That the environment plays a large part in conditioning the incidence and development of plant diseases has long been recognised. The practical difficulties inherent in the study of the effect of external factors on an exact basis, and the comparatively greater ease with which the behaviour of the causal organism in pure culture may be investigated, have, however, led plant pathologists until the last decade or so to focus their attention mainly on this latter aspect. Such information as we have on the influence of environmental conditions has largely been based on observational rather than exact experimental data. Apart, also, from the practical difficulties of the study of these factors under known controlled conditions, the problem is further complicated by the fact that the environment influences not only the parasite but also the host plant. The disease, *qua* disease, is the resultant of the activities of the host and of the parasite, and valuable information on this resultant can only be obtained from a knowledge of the two parts that go to make it up.

Yet another difficulty and one that requires intensive investigation, is the obtaining of some precise quantitative measure of "degree of attack." A mere statement of numerical percentage of diseased plants or seedlings under any given conditions, a criterion which is often taken as a measure of degree of incidence, seems to give only half the truth, expressing only the degree of absolute resistance within the limited population of plants studied. A measure is needed which will give some value to the *severity* of attack in each individual case, in terms of some physical dimension such as length or volume of diseased tissue.

Turning to the practical aspects of control of environmental conditions the factors that may be concerned can be tabulated as follows:

Physical	Chemical	Biological
Soil Temperature	Soil Nutrients	Flora and Fauna of
,, Moisture	Air, CO_2 content	the Soil
,, Aeration		
,, Reaction		
,, Condition		
Air Temperature		
,, Humidity		
,, Movement		
Light Intensity		
,, Quality		

In the study at Rothamsted of the effect of the environment in conditioning the incidence and development of the angular leaf spot disease of cotton attempts have been made to control these factors, starting with the simplest apparatus and gradually working up to apparatus in which as many factors as possible can be controlled.

The first investigation was concerned only with soil temperature in an attempt to confirm or disprove Massey's theory of the influence of this factor on primary infections of seedlings from infected seed. The work was carried out in a series of differential temperature tanks on the principle of the Ganong incubator, one end of the series being maintained at a constant high temperature (37° C.) and the other at a low temperature (13° C.), the intermediate tanks showing a range of temperatures between these extremes. Results were so conflicting that it was felt that the effect of soil temperature could not be differentiated from that of other factors, and the next step was the construction of an infection-chamber in which the entire plant could be placed under conditions of known air (and, of course, soil) temperature and humidity. With this apparatus the problem was attacked from the point of view of secondary infection, seedlings raised in the glasshouse being sprayed with a strong suspension of the organism, placed in the chamber for 48 hours, removed to the glasshouse and watched for the development of the disease. Briefly, it was found with this type of apparatus that the limiting temperature for infection with the organism was 32° C. Below this temperature the incidence was governed by the prevailing humidity. At a temperature of 28° C. infection took place with a humidity of over 70 per cent. relative saturation, but at lower humidities infection did not occur. On reducing the temperature to 25° C., however, infection could take place even at a relative humidity of 65 per cent. It is seen, therefore, that humidity and temperature are interrelated factors, the limiting humidity for infection depending on the temperature and *vice versa*.

These results were of sufficient importance for the Empire Marketing Board to take an interest in the investigation, and a grant was made for the construction of more elaborate pieces of apparatus in which it is hoped that all the factors tabulated previously may be controlled to an exact degree. They consist essentially of modified Wisconsin tanks provided with eight soil containers, and having above them glass-walled air chambers with powerful electric lights suspended close to the top. A film of water is kept flowing over the top to prevent the heat from the lamps entering the

chamber. Air temperature is controlled within a range of $1\frac{1}{2}°$ to 2° C. by means of a sensitive thermostat incorporating a bimetallic strip, the heat being provided by two carbon filament lamps at the sides of the chamber. Humidity is kept constant by a device depending on the vaporisation of water from wet muslin covering a resistance lamp enclosed in a tin through which an air stream is blown, the lamp being controlled through a relay by a hair hygrostat within the chamber. The air within the chamber is kept in circulation by a fan, and the air stream from the humidifier ensures constant changing of the atmosphere. Six of these chambers are being constructed and should be in working order early in 1929.

The paper was illustrated by lantern slides, showing the details of the several pieces of apparatus described and giving tabulated results obtained in experiments.

II. THE EFFECT OF ENVIRONMENTAL CONDITIONS ON PLANT DISEASES UNDER GLASS.

By W. F. Bewley, D.Sc.

Observant nurserymen have long realised in a broad way that the diseases attacking their plants are most serious under certain conditions of temperature and humidity, and some sort of control has been obtained by avoiding these conditions as much as possible. Observations such as these have been based largely upon the "feel" of the atmosphere on entering a house, or upon records taken with the aid of cheap and somewhat crude instruments. It is obvious that more careful observations made with accurate instruments, supplemented by experiments under properly controlled conditions must lead to vastly improved methods of control. The glasshouse industry teems with problems of this kind, and while most diseases are under some measure of cultural control, there is need for more concentrated effort before we can be reasonably satisfied with our methods.

It is only of recent years that an attempt has been made to study at all carefully the effect of physical factors upon plant diseases, and much of the pioneer work on temperature is due to the United States Department of Agriculture and Prof. L. R. Jones and his colleagues at the University of Wisconsin. This work, and subsequent papers, can be read by all who wish, and there is no need to discuss it here. It is common knowledge that the physical factors of the environment affect (1) the organism, (2) the host plant, and (3) the disease complex. The grower, whom we are trying to help, is mainly concerned with the second and third.

The effect of temperature can be seen early in the case of the tomato, especially in the first 2 months after planting. Seeds sown in December are held at a temperature of 16° C. Soil temperature at planting time is important; it should be 16° C. and certainly not lower than 14° C. At this temperature the roots continue their development in the new soil, and growth of the plant proceeds steadily. At soil temperatures below 14° C. root development is checked, some roots die, the leaves become purplish green and the growth rate is definitely retarded.

Air temperature after planting has a marked effect on the plant, as was seen during the past season in chambers where minimum night temperatures of 13° C., 16° C., 17° C., 18° C. and 21° C. were maintained by thermostatic control.

An excellent type of growth was obtained at 17° C. and 18° C. The fruit set freely and the total weight was 28 per cent. greater than that at 16° C. and 21° C. Growth at 16° C. was sturdier, darker in colour and slower, while at 13° C. the

plants were much retarded and purplish green in colour. At 21° C. growth was too rapid and soft. The plants were etiolated and the two bottom trusses failed to grow more than 1 in. in length. The plants developed "Stripe" disease very badly.

The amount of sunlight must be considered in relation to temperature. Bright sunlight matures the tissues and hardens the growth and therefore counteracts the softening effect of high temperatures. The successful cultivator of glasshouse-plants reduces the temperature in dull weather and drives his boilers in sunny weather. Sudden changes in temperature are important, especially differences between day and night temperature. This was especially noticeable this season, where, in spite of the sunny days, the night temperature has been abnormally low. The worst effect was seen in the spring when a day temperature of 41° C. followed by a 13° C. night temperature was common. As a result, the pollen did not disperse freely and the bottom trusses set badly.

The effect of environmental conditions upon certain diseases.

1. *Tomato diseases.*

Damping-off and foot rot of the tomato. Phytophthora parasitica is most dangerous during the June sowings. Many cases are recorded where seed sown in soil taken from the same heap is practically free from damping-off in December, and shows high mortality in June. This is explained by the optimum temperature of about 27° C. *Phytophthora cryptogea* has a slightly lower optimum for infection and spread, namely 24° C. *Rhizoctonia solani* has a still lower optimum at about 18° C. The damage done by all three is comparatively small below 10° C. All three require high humidities for maximum effect. Drying the surface of the box by mechanical raking helps to control "damping-off" due to *Phytophthora* but not *Rhizoctonia*.

Thielavia basicola attacks tomatoes in the pot stage and rarely continues after planting out. Infection experiments have given best results at 13° C., but temperature is not the only factor. Root infection by this fungus depends upon the growth rate: the disease spreading in the tissues most rapidly when the plant is growing slowly. Almost any method of increasing the rate of growth of the plant, will check the development of this disease.

The appearance of *Stripe disease* is governed by the state of the plant, those showing soft and rapid growth being more susceptible than more slowly growing harder plants. Abundant sunshine matures the tissues, hardens the growth and checks outbreaks of this disease. Dull weather and high humidities cause severe outbreaks. Temperatures too are important, for above an average day temperature of 24° C. Stripe fails to develop rapidly.

Sleepy disease caused by *Verticillium albo-atrum* is typically one of low temperatures and its appearance can be forecasted with uncanny accuracy by considering the temperatures during the first fortnight after planting. Temperatures between 16° C. and 24° C., with an optimum around 21° C. to 23° C., are favourable to the rapid progress of this wilt. Plants wilt at temperatures around 16° C. and recover when transferred to temperatures above 25° C. This is no doubt due to the fact that toxins capable of producing wilt are not produced by the fungus at high temperatures.

A method of cultural control applicable when a large proportion of the crop is attacked has been satisfactorily employed as follows: (1) overhead damping in place

of watering; (2) shading the houses; (3) maintaining an average temperature about 25° C.

Fusarium lycopersici rarely occurs in this country, although root rot caused by *F. oxysporum*, etc., is relatively common. Apparently the soil temperature is too low for this fungus.

2. *Cucumber diseases.*

Colletotrichum oligochaetum, which causes the dreaded "*spot*" *disease* of cucumbers, has been eradicated by house hygiene including steam sterilisation of the soil and disinfection of the superstructure with cresylic acid or formaldehyde. At the height of its activity in 1920–1923 it was held in partial check by controlling the temperature.

Investigation of the disease in the Lea Valley indicated its temperature relations as a minimum temperature of 7° C., a maximum of 30° C., with the optimum around 24° C. The rate of progress of the disease was materially checked by maintaining an average temperature above 30° C. Obviously high humidities favoured the disease.

The temperature and humidity relations of "*gummosis*" of the cucumber due to *Cladosporium cucumerinum* have not been determined, but it is known that cool, moist conditions favour its appearance. It occurs mainly during cold Springs and Autumns, and rarely during normal cucumber conditions.

The control of *Mosaic disease* is much too complicated to be achieved by environmental conditions only, but certain observations with cucumber mosaic are important commercially. The symptoms appear on leaves, flowers and fruits, but usually the fruits are not affected at temperatures below approximately 27° C. In nurseries fruit damage is obviated by suitable ventilation, which prevents the house atmosphere from rising above 27° C.

III. TEMPERATURE AND HUMIDITY IN RELATION TO TOMATO MILDEW (*CLADOSPORIUM FULVUM*).

By Mr T. Small, B.Sc.

In temperate countries tomato mildew (*Cladosporium fulvum*) is almost restricted to plants under glass, which suggests that a close relation exists between the disease and environmental conditions. Temperature and humidity are the chief factors concerned. Experiments made under controlled conditions showed that the optimum temperature for *Cladosporium fulvum* is about 22° C. Below this temperature the progress of the disease is retarded, but retardation is not appreciable until temperatures about 15° C. are reached. Such low temperatures merely check the development of the disease subsequent to infection, for severe infections take place under humid conditions at 13° C.

A study of the effect of humidity on tomato mildew showed that *C. fulvum*, in common with most fungi, is favoured by very humid conditions. At 22° C., infection is severe at 80 per cent. humidity but is rare at 70 per cent. humidity. The fungus sporulated abundantly on diseased plants exposed to 80 per cent. humidity at 20° C. but produced very few spores at 58 per cent. humidity.

Figures were quoted, calculated from continuous records, to convey some idea of the very high night humidities existing in commercial glasshouses. Experimental evidence was given to support the conclusion that the presence and persistence of such high humidities is mainly, and in some cases solely, responsible for tomato mildew.

Ordinary Meeting held at 2.30 p.m. on Friday, December 14th, in the Imperial College of Science. The President, Dr E. J. Butler, C.I.E., F.R.S., in the Chair.

THE ANTISEPTIC PRESERVATION OF WOOD[1].

By Professor Percy Groom, F.R.S.

The paper deals with felled timber and almost exclusively with preservation against fungi and thus largely excludes from consideration attacks by insects on wood.

Moreover, in all investigated cases of attack on wood by insects these cannot feed on wood without the co-operation of symbiotic protozoa, bacteria, or fungi. A number of chemical elements, especially arsenic, fluorine and mercury, provide potent preservatives against both insects and fungi attacking wood.

National economic loss is caused by the decay of wood mainly due to neglect of sanitation (*e.g.* houses) and of application of preservatives (*e.g.* mines), or by inappropriate preservative treatment (*e.g.* creosoted paving). Appropriate preservative methods can be adopted to increase the utilisation of rapidly-grown perishable timbers in temperate and tropical countries.

A sharp distinction must be drawn between mere temporary antisepsis and prolonged preservation. The duration of the protection afforded by fungicides depends upon the depth of penetration and the nature of the fungicide. Mere superficial treatment of the wood provides the shortest protection, as untreated internal wood is liable to be exposed by drying, abrasion and so forth, and the fungicide is liable to diminution by washing away, evaporation and chemical change.

Methods of application.

Preservatives may be applied to the whole of the piece of timber or solely to the part that is most vulnerable to attack: the former may be called *general* and the latter *regional* treatment.

Superficial coatings. The most ancient superficial coat is represented by the bark, and dating back to prehistoric times is the *charring* of wood by flames. *Antiseptic liquids* are applied either to prevent the germination of spores or to provide prolonged protection. Many weakly antiseptic solutions, such as sodium carbonate, serve to provide transitory protection against the germination of spores. For more prolonged preservation aqueous solutions may be used where the wood is not exposed to rain and may be applied hot or cold; but where liable to be washed away the liquid must be a tar-oil.

Excavation and slits. Even in ancient times holes were driven into the wood and preservatives were deposited in these with the object of increasing the depth of penetration. This method is now mainly used in the regional treatment of posts, the auger-holes being made just above and just below ground level.

Allied to this method is the very novel *Cobra* method, which may be described as one of *inoculation*. This is used to protect telegraph poles by treatment of freshly-

[1] As this Paper was written for and will be published in the *Empire Forestry Journal*, only a brief abstract of it is given here.

felled poles in the region that will be near the ground level. The preservative takes the form of a concentrated fungicidal paste (fluoride and 2 : 4 dinitrophenate of sodium) which is injected into the wood by means of a very large, flat, hollow inoculating needle. The "needle" merely separates the fibres, producing slits. The slits are distributed round the post for a length of a metre, but owing to the diffusion of the ingredients by the water originally in the wood the preservatives eventually form a closed protective belt round the whole of the sapwood for a length of two metres. Similar but much less extensive treatment is adopted at the top of the telegraph pole where the electric wires are fixed, and finally the pole is painted with a coat of a tar-oil preservative. The Cobra apparatus is portable.

The so-called *Boucherie* methods of impregnating wood by suction or hydrostatic pressure were considered in the paper together with modern variants of these.

Submersion. The depth of penetration ensured by submerging wood in a preservative solution varies greatly with the time of submersion, often the temperature of the solution, and the nature and condition of the wood. The wood to be treated must be seasoned. The treatment may be general or regional.

Pneumatic pressure. This was illustrated by a brief description of the ordinary method of creosoting timber and by reference to methods of economy by means of partial recovery of the creosote, or by means of an admixture of cheaper liquids.

Antiseptics.

In the past the fungicidal efficiency of liquids designed for protection of wood has been tested by experiments on cultures of various kinds of fungi on gelatine and agar media, and on wood. Misleading results have been obtained because due allowance has not been made for the fact that different species of fungi differ widely in their resistance to the same fungicide, and the fungicidal power of a given liquid on a given species of fungus may differ widely with the nutrient medium employed. Accurate results can be obtained only by testing cultures of wood-attacking fungi feeding on wood, and even in these cultures the effects of temperature and water-content intervene.

As a result of his most recent cultures on wood Prof. Richard Falck ranges fungicides in five groups, beginning with the most powerful as represented by: arsenic (arsenites and arsenates); fluorine (fluorides, silico-fluorides) and corrosive sublimate; dinitrophenol, etc.; heavy metals (copper sulphate and zinc chloride) and tar-oils.

It is evident, however, that the fungicidal efficiency of a substance does not give an accurate indication of the preservative value of this, since the substance may undergo loss by leaching out, evaporation, chemical change; or it may weaken the wood, or be dangerous by reason of its explosive or poisonous properties. Accordingly, the paper included a detailed discussion of a number of timber preservatives.

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1919 Brenchley, Miss W., D.Sc., F.L.S., F.E.S., Rothamsted Experimental Station, Harpenden, Herts.

1914 Brierley, W. B., D.Sc., F.L.S., F.R.A.I., Department of Mycology, Rothamsted Experimental Station, Harpenden, Herts.

1926 Bright, T. B., Chucks Cottage, Walton-on-the-Hill, Surrey.

1914 Brooks, F. T., M.A., F.L.S., The Botany School, Cambridge.

1921 Brooks, R. St-John, M.D., M.A., D.P.H., D.T.M. and H., Lister Institute, Chelsea Gardens, London, S.W. 1.

1924 Brown, Prof. W., M.A., D.Sc., Imperial College of Science, South Kensington, S.W. 7.

1924 Buckhurst, A. S., A.R.C.S., D.I.C., Pathological Laboratory, Milton Road, Harpenden, Herts.

1920 Buddin, W., M.A., Laboratory of Plant Pathology, University of Reading.

1922 Bunyard, G. N., 25, Bower Mount Road, Maidstone, Kent.

1920 Butler, E. J., D.Sc., C.I.E., M.B., F.R.S., F.L.S., Director, Imperial Bureau of Mycology, Kew, Surrey.

1928 Callaghan, A. R., B.Sc. Agr., Department of Agriculture, Sydney, New South Wales, Australia.

Orig. Carpenter, Prof. G. H., D.Sc., Keeper, Manchester Museum, The University, Manchester.

1927 Carroll, J., B. Agr. Sc., 110, Cook Street, Ithaca, New York State, U.S.A.

1914 Cayley, Miss D. M., John Innes Horticultural Institute, Merton, Surrey, S.W. 19.

1905 Chandler, S. E., D.Sc., F.L.S., Imperial Institute, London, S.W. 7.

1925 Cheal, W. F., Wellington House, Kirton, near Boston, Lincs.

1926 Cheesman, Prof. E. E., B.Sc., A.R.C.S., Imperial College of Tropical Agriculture, Trinidad.

1919 Chipp, Major T. F., M.C., Ph.D., D.Sc., Assistant Director, Royal Botanic Gardens, Kew, Surrey.

1908 Chittenden, F. J., F.L.S., V.M.H., Director, R.H.S. Gardens, Wisley, Ripley, Surrey.
1921 Chrystal, R. N., D.Sc., Imperial Forestry Institute, Oxford.
1905 Cornwallis*, F. S. W., Linton Park, Maidstone, Kent.
1915 Cotton, A. D., F.L.S., Royal Botanic Gardens, Kew, Surrey.
1920 Cunliffe, N., M.A., Research Institute, School of Forestry, Oxford.
1929 Curtis, Miss K. M., M.A., D.Sc., Cawthron Institute, Nelson, New Zealand.
1920 Cutler, D. W., M.A., F.L.S., Rothamsted Experimental Station, Harpenden, Herts.
1927 Dade, H. A., A.R.C.S., Mycologist, Research Branch, Department of Agriculture, Aburi, Gold Coast.
1920 Darbishire, Prof. O. V., Ph.D., B.A., F.L.S., Botany School, The University, Bristol.
1915 Davidson, J., D.Sc., F.L.S., F.E.S., The Waite Institute, University of Adelaide, S. Australia.
1927 Davies, W. Maldwyn, Ph.D., Adviser in Agricultural Zoology, University College, Memorial Buildings, Bangor, N. Wales.
1926 Davy, J. Burtt, M.A., Ph.D., F.L.S., Imperial Forestry Institute, University of Oxford.
1923 Dixon*, Miss A., M.Sc., F.R.M.S., Rothamsted Experimental Station, Harpenden, Herts.
1915 Doidge, Miss E. M., M.A., D.Sc., F.L.S., Division of Botany, Department of Agriculture, Pretoria, S. Africa.
1920 Dowson, W. J., M.A., D.Sc., F.L.S., Department of Agriculture, Hobart, Tasmania.
1920 Drummond, Prof. J. M., M.A., F.L.S., Botany School, The University, Glasgow.
1923 Du Porte, Ernest Melville, M.Sc., Ph.D., F.E.S., F.R.M.S., Macdonald College, Montreal, Canada.
1925 Durham, H. E., Sc.D., M.B., B.C., F.R.C.S., "Dunedin," Hereford.
1927 Edwards, E. E., M.Sc., Advisory Entomologist, Harper Adams Agricultural College, Newport, Salop.
1925 Ekins, E. H., B.Sc., Principal, The College, Studley, Warwickshire.
1919 Elliott, Mrs J. S. Bayliss, D.Sc., Botany School, The University, Birmingham.
1917 Eltringham, H., M.A., D.Sc., F.E.S., 6, Museum Street, Oxford.
1922 Esdaile, Miss P. C., D.Sc., F.Z.S., King's College for Women, Campden Hill Road, London, W. 8.
1927 Everett, J., B.A., Canterton Cottage, Lyndhurst, Hants.
1920 Fahmy, T., Mycological Division, Plant Protection Section, Ministry of Agriculture, Giza, Egypt.
1909 Farmer, Prof. Sir John B., M.A., D.Sc., F.R.S., LL.D., Imperial College of Science, S. Kensington, London, S.W. 7.
1920 Fenton, E. W., M.A., B.Sc., F.E.S., Biological Department, Edinburgh and East of Scotland College of Agriculture, 13, George Square, Edinburgh.
1919 Fisher, K., The School, Oundle.
1923 Fisher, R. C., B.Sc., Ph.D., Forest Products Research Laboratory, Princes Risborough, Bucks.
1922 Frew, J. G. H., M.Sc., F.Z.S., F.E.S., 111, Mawson Road, Cambridge.

1919 Fryer, C. H., 4, Manor Grove, Dry Hill, Tonbridge.
1913 Fryer, J. C. F., M.A., F.E.S., Pathological Laboratory, Milton Road, Harpenden, Herts.
1919 Fryer, P. J., F.I.C., Messrs Wm. Cooper and Nephews (Australia), Ltd., 4, O'Connell Street, Sydney, Australia.
1918 Gahan, C. J., M.A., D.Sc., F.E.S., Natural History Museum, S. Kensington, London, S.W. 7.
1914 Gardiner, Prof. J. S., M.A., F.R.S., Bredon House, Selwyn Gardens, Cambridge.
1927 Gibson, Dr W. H., The Linen Industry Research Association, The Research Institute, Lambeg, Co. Antrim.
1922 Gilchrist, Miss G. G., B.Sc., Botanical Department, The University, Bristol.
1920 Gimingham, C. T., O.B.E., F.I.C., F.E.S., Pathological Laboratory, Milton Road, Harpenden, Herts.
1920 Glynne, Miss M. D., M.Sc., F.L.S., Department of Mycology, Rothamsted Experimental Station, Harpenden, Herts.
1920 Goodey, T., D.Sc., Institute of Agricultural Parasitology, Winches Farm, Hatfield Road, St Albans.
1922 Goodwin, W., M.Sc., Ph.D., S.E. Agricultural College, Wye, Kent.
1908 Gough, G. G., B.Sc., 45, Poplar Avenue, Edgbaston, Birmingham.
1921 Gray, P. H. H., M.A., Rothamsted Experimental Station, Harpenden, Herts.
1905 Green, E. E., F.E.S., Way's End, Camberley, Surrey.
1909 Groom, Prof. P., M.A., D.Sc., F.R.S., Imperial College of Science, S. Kensington, London, S.W. 7.
1921 Grubb, N. H., M.Sc., East Malling Research Station, East Malling, Kent.
1921 Guillebaud, W. H., B.A., Forestry Commission, 22, Grosvenor Gardens, London, S.W. 1.
1929 Gurney, W. B., B.Sc., Entomologist, Department of Agriculture, Sidney, New South Wales.
1914 Güssow, H. T., F.L.S., F.R.M.S., Dominion Botanist, Central Experimental Farm, Ottawa, Canada.
1920 Gwynne-Vaughan, Prof. Dame Helen, D.B.E., D.Sc., LL.D., F.L.S., Botanical Department, Birkbeck College, Chancery Lane, London, E.C. 4.
1920 Halket, Miss A. C., B.Sc., Bedford College, Regent's Park, London, N.W.
1921 Harland, Prof. S. G., D.Sc., West Indian Agricultural College, Trinidad.
1924 Harris, R. V., Research Station, East Malling, Kent.
1927 Hatton, R. G., M.A., Director, East Malling Research Station, East Malling, Kent.
1920 Hiley, W. E., M.A., F.L.S., Research Institute, School of Forestry, Oxford.
1920 Hill, A. W., C.M.G., Sc.D., M.A., F.R.S., F.L.S., Director, Royal Botanic Gardens, Kew, Surrey.
1920 Hiscox, Miss E. R., B.Sc., Research Institute in Dairying, University College, Reading.
1924 Hockey, J. F., B.S.A., Pathologist in Charge, Plant Pathology Laboratory, Kentville, Nova Scotia, Canada.
1923 Hodson, W. E. H., A.R.C.S., D.I.C., Seale-Hayne Agricultural College, Newton-Abbot, Devon.

1920 Holden, Prof. H. S., D.Sc., F.L.S., Department of Biology, University Park, Nottingham.
1919 Horne, A. S., D.Sc., F.L.S., F.G.S., Botany School, Imperial College of Science, London, S.W. 7.
1920 Horton, E., B.Sc., F.I.C., 10, Crieff Road, Wandsworth Common, London, S.W. 18.
1927 Howes, F. N., M.Sc., Royal Botanic Gardens, Kew, Surrey.
Orig. Imms, A. D., M.A., D.Sc., F.L.S., F.E.S., Rothamsted Experimental Station, Harpenden, Herts.
1918 Jackson, Miss D. J., F.L.S., F.E.S., North Cliff, St Andrews, Fife.
1927 Jacobs, S. E., Botanical Department, Imperial College of Science and Technology, S. Kensington.
1927 James, H. C., D.Sc., c/o Agriculture Department, Scott Laboratories, Nairobi, Kenya, Africa.
1918 Jardine, N. K., Royal Botanic Gardens, Peradeniya, Ceylon.
1928 Jarrett, Miss P. H., M.Sc., Department of Mycology, Rothamsted Experimental Station, Harpenden, Herts.
1927 Jary, S. G., Advisory Entomologist, University, Reading.
1907 Jepson, F. P., M.A., F.E.S., Department of Agriculture, Peradeniya, Ceylon.
1927 Jones, G. H., M.A., Mycologist, Agricultural Department, Nigeria.
1927 Joseph, E. G., B.Sc., Imperial College of Science, S. Kensington, London, S.W. 7.
1919 Kannan, Kuhny, M.A., F.E.S., Assistant Entomologist, Government of Mysore, Bangalore, S. India.
1915 Keeble, Sir Frederick, C.B.E., M.A., Sc.D., F.R.S., c/o Nitram, Ltd., 28–30, Grosvenor Gardens, S.W. 1.
1920 Kidd, F., M.A., D.Sc., Low Temperature Research Station, Downing Street, Cambridge.
1907 King, H. H., F.L.S., F.E.S., Government Entomologist, Wellcome Tropical Research Laboratories, Khartoum, Sudan.
1907 King, Prof. L. A. L., M.A., West of Scotland Agricultural College, 6, Blythewood Square, Glasgow.
1926 Kingston, H. T., 8, Sewardstone Road, Waltham Abbey, Essex.
1924 Knight, R.C., D.Sc., Assistant Director, Research Station, East Malling, Kent.
1921 Lacey, Miss M. S., D.Sc., Botanical Department, Imperial College, London, S.W. 7.
1921 Laurie, Prof. R. D., M.A., F.Z.S., University College of North Wales, Aberystwith.
1908 Lees, A. H., M.A., National Fruit and Cider Institute, Long Ashton, Bristol.
1909 Leiper, Prof. R. T., M.D., F.R.S., School of Tropical Medicine, Endsleigh Gardens, Euston Road, London, W.C. 1.
1926 Le Pelley, R. H., Ph.D., School of Agriculture, Cambridge.
1915 Lister, A. B., B.Sc., D.I.C., Experiment Station, Cheshunt, Herts.
1920 Lloyd, Llewellyn, D.Sc., Azare, Kano, Nigeria, Africa.
1914 McClellan, F. C., C.B.E., M.R.A.C., F.L.S., Director of Agriculture, Zanzibar.
1928 McCulloch, R. F., B.Sc. (Agr.), School of Rural Economy, Oxford.

Orig. MacDougall*, Prof. R. S., M.A., D.Sc., F.E.S., F.R.S.E., 9, Dryden Place, Edinburgh.

1925 Macleod, D. J., M.A., Officer in Charge, Dominion Plant Pathological Laboratory, Fredericton, New Brunswick.

1927 McLellan, Miss Ethel, D.Sc., Botanical Department, The University, Melbourne, Australia.

1909 Mangan, Prof. J., M.A., University College, Galway, Ireland.

1920 Mangham*, Prof. S., M.A., Botany Department, University College, Southampton.

1917 Mann, H. H., D.Sc., F.L.S., 28, Ashburton Road, Oxton, Birkenhead.

1914 Marshall, G. A. K., D.Sc., C.M.G., F.R.S., F.Z.S., F.E.S., Director, Imperial Bureau of Entomology, Natural History Museum, London, S.W. 7.

1927 Martley, J. F., A.R.C.S., Forests Products Research Laboratory (Oxford Branch), c/o Imperial Forestry Institute, Oxford.

1922 Mason, E. W., M.A., M.Sc., Imperial Bureau of Mycology, 17, Kew Green, Surrey.

1920 Mason, F. A., 29, Frankland Terrace, Leopold Street, Leeds.

1921 Mason, T. G., M.A., Sc.D., Cotton Research Station, Trinidad.

1927 Massee, A. M., East Malling Research Station, East Malling, Kent.

1920 Mattick, A. T. R., B.Sc., Research Institute in Dairying, University College, Reading.

1923 Miles, H. W., B.Sc., Department of Agricultural Entomology, University of Manchester.

1921 Millard, Prof. W. A., D.Sc., Department of Agriculture, The University, Leeds.

1928 Moore, W. C., M.A., Plant Pathological Laboratory, Milton Road, Harpenden, Herts.

1922 Morland, D. M. T., M.A., Rothamsted Experimental Station, Harpenden, Herts.

1920 Morris, H. M., M.Sc., Agricultural Department, Nicosia, Cyprus.

1920 Mosley, F. O., F.L.S., Pathology Laboratory, Messrs Lowe and Shawyer, Uxbridge.

1927 Muir, F. A. G., D.Sc., The Den, Farnham Common, Bucks.

1925 Mumford, E. Philpott, B.Sc., Christ's College, Cambridge.

1914 Munro, J. W., M.A., D.Sc., Entomology Department, Imperial College of Science, S. Kensington, London, S.W. 7.

1919 Murphy, A. J., 2, Dorset Square, London, N.W. 1.

1920 Murphy, Prof. P. A., D.Sc., B.A., Albert Agricultural College, Glasnevin, Dublin, Ireland.

1925 Muskett, A. E., B.Sc., A.R.C.S., The Queen's University, Belfast.

1914 Neave, S. A., M.A., D.Sc., F.Z.S., F.E.S., Assistant Director, Imperial Bureau of Entomology, 41, Queens Gate, London, S.W. 7.

1927 Nelson, A., Ph.D., B.Sc., Superintendent of Research, Department of Agriculture, Hobart, Tasmania.

1927 Nirula, R. L., B.Sc., Ph.D., D.I.C., Ghangwul, P.O. Jhawrian, District Shahpore, Punjab, India.

1923 Noel, Miss E. F., 37, Moscow Court, London, W. 2.

1920 Nowell, W., D.I.C., F.L.S., Director, Research Institute, Amani, Tanga, Tanganyika.
1923 Ogilvie, L., M.A., B.Sc., M.Sc., Research Station, Long Ashton, Bristol.
1925 Oldham, J. N., B.Sc., Institute of Agricultural Parasitology, Winches Farm, Hatfield Road, St Albans.
1923 Owen, O., B.Sc., A.I.C., Experimental and Research Station, Cheshunt, Herts.
1919 Paine, S. G., D.Sc., F.I.C., Imperial College of Science, London, S.W. 7.
1920 Palmer, R., "Standeford," Baldock Road, Letchworth.
1920 Parker, T., Advisory and Research Department, Messrs Abol, Ltd., Beltring, Paddock Wood, Kent.
1914 Pearson, J., D.Sc., Director of the Museum, Colombo, Ceylon.
1919 Percival, Prof. J., M.A., Sc.D., F.L.S., University, Reading.
1914 Petherbridge, F. R., M.A., School of Agriculture, Cambridge.
Orig. Pethybridge, G. H., M.A., M.R.I.A., Ph.D., B.Sc., Pathological Laboratory, Milton Road, Harpenden, Herts.
1919 Pomeroy, A. W. J., Government Entomologist, Medical Research Institute, P.O. Box 300, Accra.
1915 Porter*, A., D.Sc., S. African Institute for Medical Research, P.O. 1038, Johannesburg.
1907 Poulton*, Prof. E. B., M.A., D.Sc., LL.D., F.R.S., Wykeham House, Oxford.
1919 Prain*, Sir David, Lt-Col., C.M.G., C.I.E., M.A., M.B., F.R.S., LL.D., F.R.S.E., V.M.H., The Well Farm, Warlingham, Surrey.
1923 Preston, N. C., B.Sc., Harper Adams Agricultural College, Newport, Salop.
1908 Priestley, Prof. J. H., D.S.O., B.Sc., Botany School, The University, Leeds.
1928 Ramsbottom, J., O.B.E., M.A., British Museum (Natural History), Cromwell Road, London, S.W. 7.
1927 Rege, R. D., Ph.D., Institute of Plant Industry, Indore, Central India.
1921 Richards, E. H., B.Sc., F.I.C., Rothamsted Experimental Station, Harpenden, Herts.
1921 Roach, W. A., B.Sc., A.R.C.S., D.I.C., A.I.C., East Malling Research Station, East Malling, Kent.
1919 Roberts, A. W. Rymer, M.A., F.E.S., Molteno Institute for Research in Parasitology, Cambridge.
1923 Robinson, D. H., B.Sc., Harper Adams Agricultural College, Newport, Salop.
1916 Robinson, Prof. W., D.Sc., Stoneleigh, Caradoc Road, Aberystwyth.
1918 Robson, R., Institute of Agriculture, Chelmsford.
1920 Roebuck, A., N.D.A., Midland Agricultural College, Sutton Bonnington, Derbyshire.
1919 Russell, Sir John, D.Sc., F.R.S., Director, Rothamsted Experimental Station, Harpenden, Herts.
1914 Salmon, Prof. E. S., F.L.S., S.E. Agricultural College, Wye, Kent.
1923 Samuel, G., B.Sc., University of Adelaide, S. Australia.
1920 Sandon, H., M.A., Rothamsted Experimental Station, Harpenden, Herts.
1921 Sargent, R. H., Technical College, Darlington.
1919 Searle, G. O., B.Sc., Linen Industry Research Association, Glenmore House, Lambeg, Co. Antrim, Belfast.
1923 Singh, Bhai Inder, 6, Tapp Road, Lahore, India.

1920 Small*, Prof. J., D.Sc., F.L.S., Department of Botany, Queen's University, Belfast.
1920 Smith, E. Holmes, B.Sc., Botany School, The University, Manchester.
1925 Smith, F. E. V., B.Sc., Microbiologist, Department of Agriculture, Kingston, Jamaica, British W. Indies.
1920 Smith, J. Henderson, M.B., Ch.B., B.A., Department of Mycology, Rothamsted Experimental Station, Harpenden, Herts.
1920 Smith, K. M., A.R.C.S., D.I.C., School of Agriculture, Cambridge.
1927 Smith, Prof. N. J. G., M.A., B.Sc., Ph.D. (Camb.), Rhodes University College, Grahamstown (Cape), S. Africa.
1922 Snell, Mrs E., Solent Court Farm, Warsash, Hants.
1913 South, F. W., M.A., Agricultural Department, Kuala Lumpur, Federated Malay States.
1928 Steer, W., B.A., East Malling Research Station, East Malling, Kent.
1925 Stell, F., Department of Agriculture, Port of Spain, Trinidad, British West Indies.
1919 Speyer, E. R., M.A., Ridgehurst, Shenley, Herts.
1920 Spinks, G. T., M.A., Research Station, Long Ashton, Bristol.
1923 Staniland, L. N., A.R.C.S., D.I.C., Research Station, Long Ashton, Bristol.
1920 Stapledon, Prof. R. G., M.A., Agricultural Buildings, Alexandra Road, Aberystwyth.
1921 Stenton, R., F.E.S., Milton Road, Harpenden, Herts.
1922 Stirrup, H. H., M.Sc., Midland Agricultural College, Sutton Bonnington, Loughborough, Derby.
1919 Stone, H., Forestry School, Cambridge.
1926 Storey, H. H., M.A., Ph.D., East African Research Station, Amani, Tanga, Tanganyika.
1927 Stoughton, R. H., B.Sc., A.R.C.S., F.L.S., Department of Mycology, Rothamsted Experimental Station, Harpenden, Herts.
1921 Sutton, E. C. F., Sidmouth Grange, Earley, near Reading.
1919 Sutton, M. H., F.L.S., Erlegh Park, Whiteknights, Reading.
1905 Swanton, E. W., A.L.S., Educational Museum, Haslemere, Surrey.
1919 Tabor, R. J., B.Sc., Botany School, Imperial College of Science, London, S.W. 7.
1921 Tattersfield, F., D.Sc., F.I.C., Rothamsted Experimental Station, Harpenden, Herts.
1915 Taylor, H. V., A.R.C.S., B.Sc., Ministry of Agriculture, 10, Whitehall Place, London, S.W. 1.
1928 Taylor, T. H., M.A., Department of Agriculture, The University, Leeds.
1927 Thaysen, A. C., Ph.D., Bacteriological Laboratory, Royal Naval Cordite Factory, Holton Heath, Dorset.
1928 Thomson, W. S., B.A., Empire Marketing Board, 2, Queen Anne's Gate Buildings, London, S.W. 1.
1919 Thornton, H. G., B.A., Rothamsted Experimental Station, Harpenden, Herts.
1920 Tillyard, R. J., D.Sc., M.A., Sc.D., F.R.S., F.E.S., Commonwealth Entomologist, Canberra, Australia.
1927 Tincker, M. A., M.A., M.Sc., Royal Horticultural Society's Gardens and Laboratory, Wisley, Ripley, Surrey.

1919 Trow, Principal A. H., D.Sc., F.L.S., University College, Cardiff.
1927 Turner, W. H., B.Sc., Technical Department, Geo. Monro, Ltd., Waltham Cross.
1913 Urich, F. W., Imperial College of Tropical Agriculture, Trinidad, British West Indies.
1921 Voelcker, J. A., M.A., B.Sc., Ph.D., F.I.C., 1, Tudor Street, London, E.C. 4.
1920 Wakefield, Miss E. M., M.A., F.L.S., Royal Botanic Gardens, Kew, Surrey.
1923 Walkden, H., The Raft, Derbyshire Road, Sale, Manchester.
1919 Waller, J. C., B.A., Physiological Department, The University, Liverpool.
Orig. Warburton, C., M.A., Yew Garth, Grantchester, Cambridge.
1913 Wardle, R. A., M.Sc., Zoological Department, The University, Manchester.
1919 Ware, W. M., B.Sc., S.E. Agricultural College, Wye, Kent.
1922 Warington, Miss K., M.Sc., Rothamsted Experimental Station, Harpenden, Herts.
1914 Waterston, J., M.A., D.Sc., Natural History Museum, London, S.W. 7.
1920 Watt, A. S., B.A., Forestry Department, The University Botanic Gardens, Aberdeen.
1919 Weiss, Prof. F. E., D.Sc., F.R.S., F.L.S., Botany School, The University, Manchester.
1918 West, C., D.Sc., A.R.C.S., D.I.C., F.L.S., 7, Colfe Road, Forest Hill, London, S.E. 23.
1923 Weston, W. A. R. Dillon, M.A., School of Agriculture, Cambridge.
1921 Whitehead, T., D.Sc., A.R.C.S., University College of North Wales, Memorial Buildings, Bangor.
1912 Williams, C. B., B.A., Research Institute, Amani, Tanga, Tanganyika.
1920 Williams, Prof. R. Stenhouse, M.B., C.M., B.Sc., D.P.H., Research Institute in Dairying, University College, Reading.
1927 Williams, T. L., B.A., A.I.C.T.A., Botanist, Agricultural Research Branch, Aburi, Gold Coast.
1909 Williamson, H. C., M.A., D.Sc., Pacific Biological Station, Namaimo, B.C., Canada.
1919 Willis, J. C., M.A., Sc.D., F.R.S., F.L.S., 8, Cavendish Avenue, Cambridge.
1923 Wilson, Miss A. P., A.R.C.S., Horticultural College, Swanley, Kent.
1920 Wilson, G. Fox, R.H.S. Gardens, Wisley, Ripley, Surrey.
1914 Wilson, M., D.Sc., R.B.S., A.R.C.S., Royal Botanic Gardens, Edinburgh.
1921 Wiltshire, S. P., B.A., B.Sc., Assistant Director, Imperial Bureau of Mycology, 17, Kew Green, Kew, Surrey.
1921 Woodcock, G. S., address not known.
1926 Woodward, R. C., M.A., School of Rural Economy, Parks Road, Oxford.
1914 Wormald, H., D.Sc., A.R.C.S., East Malling Research Station, East Malling. Kent.
1920 Wortley, E. J., F.I.C., M.B.E., F.C.S., Director of Agriculture, Zomba, Nyassaland.

LAWS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

I. The Association shall be called "The Association of Economic Biologists."

II. The object of the Association shall be to promote the study and advancement of all branches of Biology with especial reference to their applied aspects.

III. The Association shall consist of Ordinary and Honorary Members.

IV. Each candidate for ordinary membership shall be a subject of the British Crown. The nomination form of each candidate for ordinary membership shall bear the signatures of two members and shall be forwarded to the Secretaries. The nomination shall be submitted to the Council and, if approved, the election of the candidate shall be recommended to the Association at the next General Meeting. For the election of any candidate two-thirds of the votes of the members present and voting shall be cast in favour of the candidate.

V. All ordinary members on first election shall pay an entrance fee of half-a-guinea. Ordinary members shall pay an annual subscription of twenty-five shillings, due on January 1st of each year, or may compound for their subscriptions by payment of a sum of twenty-five pounds.

VI. Every member elected to the Association shall receive notice to that effect from the Secretaries and shall continue a member until his written resignation shall be received by the Secretaries, or until his membership be forfeited under the laws. (A member shall be liable for the annual subscription for the year in which his resignation takes effect and, notwithstanding his resignation, shall, if he so desires, receive any subsequent publications of the Association issued during that year.)

VII. Ordinary members shall be entitled to admission to all the meetings of the Association, to vote thereat, to present papers, to take part in discussions, and to receive a copy of the Association's publications. Each member shall be entitled personally to introduce non-members to any General Meeting of the Association. But no member whose subscription is in arrears shall be entitled to vote at a General Meeting or to receive the Association's publications, nor shall any publication be sent to a new member until his entrance fee and subscription shall have been received.

The Council may remove from the roll of the Association any member whose subscription is one year or more in arrears.

VIII. Honorary Members shall be persons, not subjects of the British Crown, who have contributed to an eminent degree to the advancement of the Science of Applied Biology. They shall be recommended by a majority of the whole Council and elected in the same manner as Ordinary Members. The number of Honorary Members shall not exceed twelve and not more than two shall be elected in any one year.

Honorary Members shall each receive a copy of the Association's publications and shall not be liable for the payment of an entrance fee or annual subscription.

Their privileges shall be the same as those of Ordinary Members except that they shall not be entitled to vote at any election or meeting of the Association.

IX. The business of the Association shall be conducted by a Council consisting of a President, a Treasurer, the Secretaries, of whom there shall be two; one representing the Botanical, the other the Zoological Sections of the Association, the Editors of the Journal, of whom there shall be two, and twelve Ordinary Members. Two members of the Council shall be nominated by the President to act as Vice-Presidents.

X. All properties of the Association, both present and future, shall be deemed to be vested in the Council of the Association for the time being, in conformity with the provisions of the Literary and Scientific Institutions Act, 1854.

XI. The Council shall meet at such times as they may determine; six members shall form a quorum.

XII. The Council shall have the power to fill any vacancies among its number that may occur other than those resulting from the selection for annual retirement from the Council referred to in Law XVII.

XIII. The Council shall have power, at any of their meetings, by two-thirds of the votes of those present and voting, to recommend the removal from the roll of membership of the name of any member for the reason that in their opinion it is contrary to the interests of the Association that he shall remain a member. Such recommendation shall be submitted to the Association at the next General Meeting. For the ejection from the Association of any member two-thirds of the votes of the members present and voting shall be cast in favour of such ejection.

XIV. The Council shall appoint a Publication Committee consisting of the Editors, the Treasurer, two Ordinary Members of the Council, and two Ordinary Members of the Association, who shall be responsible for the publication of the Journal of the Association.

XV. The Council, at a meeting prior to the Annual General Meeting, shall appoint one or more Auditors to audit the Treasurer's accounts.

XVI. The Council shall purchase such books, instruments, specimens, furniture and other necessaries as may be required, pass the accounts and authorise their payment, and generally manage the affairs and administer the funds of the Association.

XVII. At a meeting prior to the Annual General Meeting the Council shall elect fourteen members of the existing Council and four members of the Association, not members of the existing Council, whom they recommend to the Association for election into the Council for the ensuing year. Any member of the Council vacating office shall not be eligible for re-appointment as an ordinary member of Council until after the lapse of twelve months. The list as drawn up by the Council shall be sent to all members resident in Great Britain and Ireland at least four weeks before the date of the Annual General Meeting. It shall be competent for any member, on receipt of the recommendations of the Council, to add the name of a member or members of the Association to the list of candidates for election to the Council; such additional nominations, duly seconded, must be in the hands of one of the Secretaries not less than fourteen days before the Annual General Meeting.

The Secretaries shall when necessary, and not less than seven days before the Annual General Meeting, issue to every member of the Association resident in Great Britain and Ireland a completed list of the proposed Officers and Council for the year, indicating the names of the proposers and seconders of candidates other than the Council's nominees.

The election of new Officers and Council shall be conducted in the following manner:—At the Annual General Meeting each member present shall receive the list of Officers and Council proposed for the year. If no additional nominations have been received after the Council's recommendations, the list shall be put to the meeting and voted on by a show of hands and the result declared by the Chairman. If additional nominations have been received a ballot shall be taken; each member voting shall hand in person to one of the Secretaries a copy of the list on which has been indicated the names of those candidates whom the member voting desires to serve on the Council. When the ballot has been declared closed the Chairman shall appoint from among the members present, two persons, not candidates for election, to serve as Scrutineers. In examining the lists handed in the Scrutineers shall set aside and take no account of any ballot paper which supports candidates for more than the number provided for in Law IX, nor of any ballot paper which indicates the identity of the member voting. The Scrutineers shall report to the Chairman of the meeting the result of their scrutiny, and the Chairman before the close of the meeting, shall announce the result of the ballot. In the case of an equality of votes for any candidates, the power of selection between them shall rest with the Chairman of the meeting and shall be exercised before announcing the result of the ballot.

XVIII. The Association shall meet at times and places to be decided by the Council.

At all Ordinary General Meetings ten shall form a quorum (see also Law XIX). All meetings shall be announced by circular addressed to each member resident in Great Britain and Ireland. At all Ordinary General Meetings the order of business shall be decided by the Chairman.

An Annual General Meeting shall unless otherwise decided by the Council be held at the date of the Ordinary General Meeting falling nearest to the beginning of the year.

At this General Meeting the order of business shall be:—

1. The reading of the minutes of the previous meeting.
2. The reading of a report of the Council on the work of the past year.
3. The statement of the Treasurer.
4. The election of Members.
5. The election of Officers and other Members of the Council.
6. Other business.

A Special General Meeting may be called to discuss or take action upon any matter affecting the interests of the Association.

A Special General Meeting shall be called either by the decision of the Council or at the request of at least ten members addressed to the Secretaries.

XIX. No new law shall be passed nor any standing law altered or added to, nor any other change in the constitution of the Association made except by a Special

General Meeting of which for this purpose a fourteen days' notice must be sent to all Members resident in Great Britain and Ireland.

The requisition for such a Special General Meeting duly signed and stating in writing the laws proposed or the alteration desired, must be delivered to one of the Secretaries, who shall within a reasonable period call such a meeting. The proposed new laws or alterations in the laws shall be printed in the circular convening the meeting.

At a Special General Meeting convened for the purpose of altering the constitution or amending the laws, fifteen shall form a quorum and no motion can be passed except by a two-thirds majority of those present and voting.